KINETICS OF SWINE WASTE ASSIMILATION BY PHOTOTROPHIC SULFUR BACTERIA

By

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This dissertation is dedicated to the loving memory of my parents, Stanford and Eunice Earle, whose vision, sacrifice, and guidance during my formative years were instrumental in the accomplishment of this work.

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The degree of waste treatment achieved in anaerobic processes is usually much lower than that of aerobic biological processes. However, anaerobic waste stabilization is accompanied by the evolution of valuable gases as end products. Use of anaerobic photobiological processes for waste stabilization has not been exploited, although their potential for such application has been recognized for some time.

Among the metabolic end products of anaerobic processes, are the gases hydrogen, hydrogen sulfide, and ammonia. Several of these potentially toxic end products are readily assimilated by phototrophic bacteria under suitable conditions.

In a series of bench-scale continuous culture studies, the anaerobic processing of swine waste under illuminated

conditions was investigated. Two daily fed, completely-mixed 4.0 L reactors, one illuminated (ER) and the other nonilluminated (CR), were inoculated with phototrophic bacterial culture and operated at retention times of 5, 7, 8.5, 10, 15, 20, and 30 days until achievement of steady-state conditions or washout of the phototrophic bacteria had occurred. All experiments were conducted at a controlled temperature of 27 $\pm$ 1°C, using waste with a volatile solids concentration of 1.0 $\pm$ 0.1 percent. Kinetic parameters were determined for bacterial growth, biomass productivity, and substrate uptake. The reaction rate coefficient was found to be 0.200 L/g-day.

Presence of phototrophic bacteria enhanced the waste treatment capability of the anaerobic digestion process, achieving reductions of 84 to 90 percent in soluble COD levels, and 66 to 74 percent in soluble Kjeldahl-N levels. Uptake of ammonia-N and soluble P was 68 percent and 46 percent, respectively. Minimum biological solids retention time was determined to lie between 8.5 and 10 days.

Specific methane yield was 0.173 L/L vol-d in the ER and 0.181 L/L vol-d in the CR. The methane content of the biogas from the ER ranged from 64 to 71 percent. Biomass productivity and waste treatment were optimized at 15-days SRT.

CHAPTER 1
INTRODUCTION

1.1 Problems Created By Animal Wastes

Improper management of the high-strength wastes generated by agricultural operations, including feedlots, has frequently resulted in the overloading and pollution of rivers, streams, and lakes, with other adverse consequential impacts on the environment. In reports submitted to the Environmental Protection Agency (EPA) (182) in 1977, agricultural sources accounted for 93 of 503 fish-kills. Of this number, manure-silage drainage was directly implicated in 32 incidents.

With the continued trend towards industrialization of livestock farming, effective and efficient disposal of the large volumes of highly concentrated waste generated under confined conditions each day, therefore, becomes a problem of major proportions (99,129,191). Traditionally, such wastes have been disposed of by land-spreading to serve as soil conditioner and fertilizer. The increased volume of collectible waste generated under confined conditions, coupled with the reduction in area available for land-disposal, now makes this method unsuitable for such operations.

In a 1978 survey (181), it was indicated that approximately 158 million metric tonnes of dry animal manure was produced annually in the United States. Confined livestock operations accounted for 61 million tonnes (39 percent). Of this latter volume, swine operations contributed a total of 5.5 million tonnes (9 percent). The annually-produced volume of waste contains approximately 7.0 million tonnes of nitrogen, 1.7 million tonnes of phosphorus, and 3.8 million tonnes of potassium, indicating the pollutional potential of the waste.

The environmental impact of these wastes is evident, not only in their observed effects on waterways, but also in the atmosphere. Various gases are produced by microbial degradation of stored wastes. These include hydrogen sulfide (H_2S), ammonia (NH_3), carbon dioxide (CO_2), and methane (CH_4) (126,165). Of these gases, H_2S and NH_3 may be toxic to both man and livestock and are also associated with odor offensiveness. Odors have been observed to increase with increasing concentrations of volatile fatty acids (VFA), phenol, p-cresol and skatole (196). Other recorded contributors to air pollution and offensive odor of animal waste slurries include methanethiol, dimethyl sulfide, diethyl sulfide, propyl acetate, n-butyl acetate, trimethylamine, and ethylamine (194).

1.2 Waste Management Considerations

Conventional techniques of municipal waste management are not appropriate for the very highly concentrated livestock wastes which are encountered. Swine wastes may have chemical oxygen demand (COD) values in excess of 80 000 mg/L, and biochemical oxygen demand (BOD_5) values in excess of 30 000 mg/L, compared with 350-450 mg/L, and 250-300 mg/L, respectively, for municipal waste. The options available for the management of livestock wastes are (1) utilization and (2) treatment and disposal. Wastes may be used as plant nutrients (157,195), as feed ingredients for farm animals and fish (69,79,200,205), as a substrate for microbial and insect protein synthesis (16,28,118,124), and as a substrate for microbial methane production (51,85,168).

Waste treatment and disposal techniques are largely dependent on the characteristics of the waste. These characteristics are, in turn, influenced by animal-type, feed-type, and method of confinement. Available waste treatment and/or disposal techniques include composting (153,169), dehydration and incineration (47), use of oxidation ditches or other aeration processes (56,57), photosynthetic reclamation (89,91,146,150), facultative and anaerobic lagoons (62,125,193), and anaerobic digestion (168). Of these, anaerobic processes are most commonly used in the management of swine waste.

1.3 Anaerobic Processing of Animal Wastes

Application of anaerobic biotechnology to the stabilization of organic solids and the treatment of highly concentrated liquid wastes has been investigated and implemented for several years (70,148,201). Wide interest in the development of this naturally-occurring stabilization process for waste treatment was stimulated at the beginning of the twentieth century. Early interest was in conventional anaerobic digestion as a stabilization process for sewage sludge and for the generation of methane gas. Currently, the technology is being investigated for general application to waste treatment problems.

Because of the high total solids (TS) and BOD_5 concentrations of swine waste, anaerobic digestion has been the preferred method of processing where energy recovery in the form of methane has been a prime consideration (63,72,199, 207). Similar production of energy does not occur in aerobic waste treatment processes. Several studies on swine waste digestion have been conducted (66,74,204), and the literature contains numerous references to the advantages and disadvantages of the process (19,33,73). The main advantages of the process are production of a useful product in the form of methane gas, and low levels of microbial cells. Referenced disadvantages include high initial capital outlay, high operation and maintenance costs, and

process instability (13,25,104). In addition, a high degree of treatment usually is not achieved by this method.

The two types of digestion systems commonly in use today are the conventional or standard rate digester (71,105), which is used primarily for the stabilization of thicker sludges, and the anaerobic filter or fixed bed reactor (184,206)), used for the treatment of more dilute or settled wastes. In standard rate digestion, the digester may either be mixed or unmixed, heated or unheated, and it usually is operated at retention times in excess of 10 days. Operating temperature may be within the mesophilic or thermophilic range, but the majority of digesters are operated within the mesophilic range, usually at a temperature of 35-37° C. Fixed bed reactors are packed with a solid medium, such as wood chips, to which the bacterial cells attach. Washout of cells is thereby minimized and consequently liquid retention times may be reduced.

Because of the very long retention times which are possible, anaerobic waste stabilization ponds, which have been applied to the disposal of swine waste for several years (62,125,193), provide operators with an inexpensive but very effective alternative to anaerobic digestion. The initial capital outlay required is low, and such stabilization ponds are virtually free of operation and maintenance costs. However, they suffer the disadvantage of being odorous at times. Indications are that the odors emanating

from such lagoons can be eliminated by encouraging the development of phototrophic anaerobes (7).

1.4 Application of Phototrophic Sulfur Bacteria to Waste Treatment Problems

Waste stabilization ponds receiving municipal, industrial, or agricultural wastes, and exhibiting anaerobic characteristics, are often distinctly colored by a large population of phototrophic sulfur bacteria (39,40,110,116). Successful efforts have been made to study and apply these bacteria to the treatment of certain wastes under controlled conditions. These bacteria are particularly useful in situations where wastes containing high levels of sulfide must be treated. This is the case with fellmongery wastes resulting from the unhairing of hides prior to tanning. These wastes are highly saline, have pH levels of 12 to 13, and contain sulfide concentrations ranging from 80 to over 400 mg/L. Other waste constituents include insoluble organics, hydrosulfides, thiosulfates, and chlorides of sodium, calcium, and ammonium, as well as free ammonia (38). Effective treatment of these wastes has been achieved in lagoon systems designed for utilization of purple sulfur bacteria (111,112).

A variety of industrial wastes with high BOD_5 levels (2000 to greater than 10,000 mg/L) have been successfully treated in photobiological treatment plants utilizing phototrophic bacteria and algae. These include wastes from the

starch, woolwashing, canned food, and pharmaceutical industries (88,90). It has been indicated that purple sulfur bacteria produce substances which inactivate some animal and human pathogenic viruses (88). Successful removal of amines (putrescene and cadaverine) was also noted. Use of phototrophic bacteria in the treatment of hazardous wastes (86), sewage sludge and cattle feedlot effluent (173), and the effluent from anaerobic waste treatment systems has also been reported (87).

In lagoons in which these microorganisms proliferate, a marked reduction in odors has been noted (7,39,189). This has been attributed to photosynthetic metabolism of the phototrophic sulfur bacteria, in which H_2S is used as electron donor for photosynthesis and consequently is oxidized to elemental sulfur. The phototrophic sulfur bacteria are divided into two groups, purple sulfur bacteria and green sulfur bacteria (139). The majority of purple sulfur bacteria store elemental sulfur internally, whereas the green sulfur bacteria deposit sulfur externally. Studies on lagoons treating organic industrial wastes (40,90,110) have confirmed that, in addition to oxidizing inorganic sulfur compounds, phototrophic sulfur bacteria utilize a number of metabolic end products which would otherwise accumulate under anaerobic conditions, with negative effects. Notable among these are certain organic acids.

In the management of swine wastes, the coupling of anaerobic digestion with accelerated photosynthetic systems offers operators an attractive option for waste treatment and fuel and feed production. The photosynthetic stage may involve the use of phototrophic sulfur bacteria as detoxifiers (88,90) followed by algal cultures. By their ability to oxidize sulfide, the bacteria remove a primary toxicant and thus condition the medium for growth of the algae. Both the bacterial and algal cells may then be harvested and used as a protein source. The protein content of the bacterial cells is reported to be in excess of 70 percent (84,179).

In a series of laboratory-scale batch studies, purple sulfur bacteria were cultured in a swine waste medium (45). From these studies it was concluded that the presence of these microorganisms was advantageous to the anaerobic treatment process. It is therefore suggested that the design of waste treatment systems incorporating phototrophic sulfur bacteria could result in enhanced treatment of highly concentrated organic wastes. Before such design can be undertaken, the kinetic parameters influencing growth and substrate uptake by these bacteria, in mixed undefined culture as commonly observed in waste stabilization lagoons, must be determined.

1.5 Research Objectives

This laboratory-scale research project was designed to define the kinetic parameters which influence the growth and substrate uptake of phototrophic sulfur bacteria. The specific objectives were to

1. Conduct laboratory-scale, continuous culture anaerobic studies with phototrophic sulfur bacteria and determine their impact on waste treatment.

2. Determine the kinetic parameters which influence the anaerobic degradation of swine waste by these microorganisms, and the loading rate and retention time required for optimum treatment.

3. Assess the effect of phototrophic sulfur bacteria on biogasification in the anaerobic digestion process.

4. Develop a mathematical model for waste degradation and biomass production in a waste treatment system incorporating phototrophic sulfur bacteria.

CHAPTER 2
LITERATURE REVIEW

2.1 Mechanism of Anaerobic Decomposition

In natural aquatic systems, the decay of organic matter occurs either aerobically, in the presence of oxygen, or anaerobically, in the absence of oxygen. These processes are mediated by aerobic, facultative, or anaerobic microorganisms which degrade the organic matter, producing new cell mass, maintenance energy and stabilized end products. The anaerobic cycle of decomposition in nature, with emphasis on the elements carbon, nitrogen and sulfur, is illustrated in Figure 2-1 (117). The stabilized end products of this cycle are methane (CH_4), carbon dioxide (CO_2), and humus.

In biological waste treatment systems, the environment of microorganisms is controlled to achieve optimum metabolic activity, resulting in maximum stabilization of organic matter. In such systems, as well as in nature, this stabilization is accomplished by a combination of two metabolic processes, oxidation and synthesis. In aerobic processes, dissolved oxygen is the ultimate hydrogen acceptor, whereas in anaerobic processes the ultimate hydrogen acceptor may be oxidized organic matter, nitrates, nitrites, sulfates or carbon dioxide (CO_2).

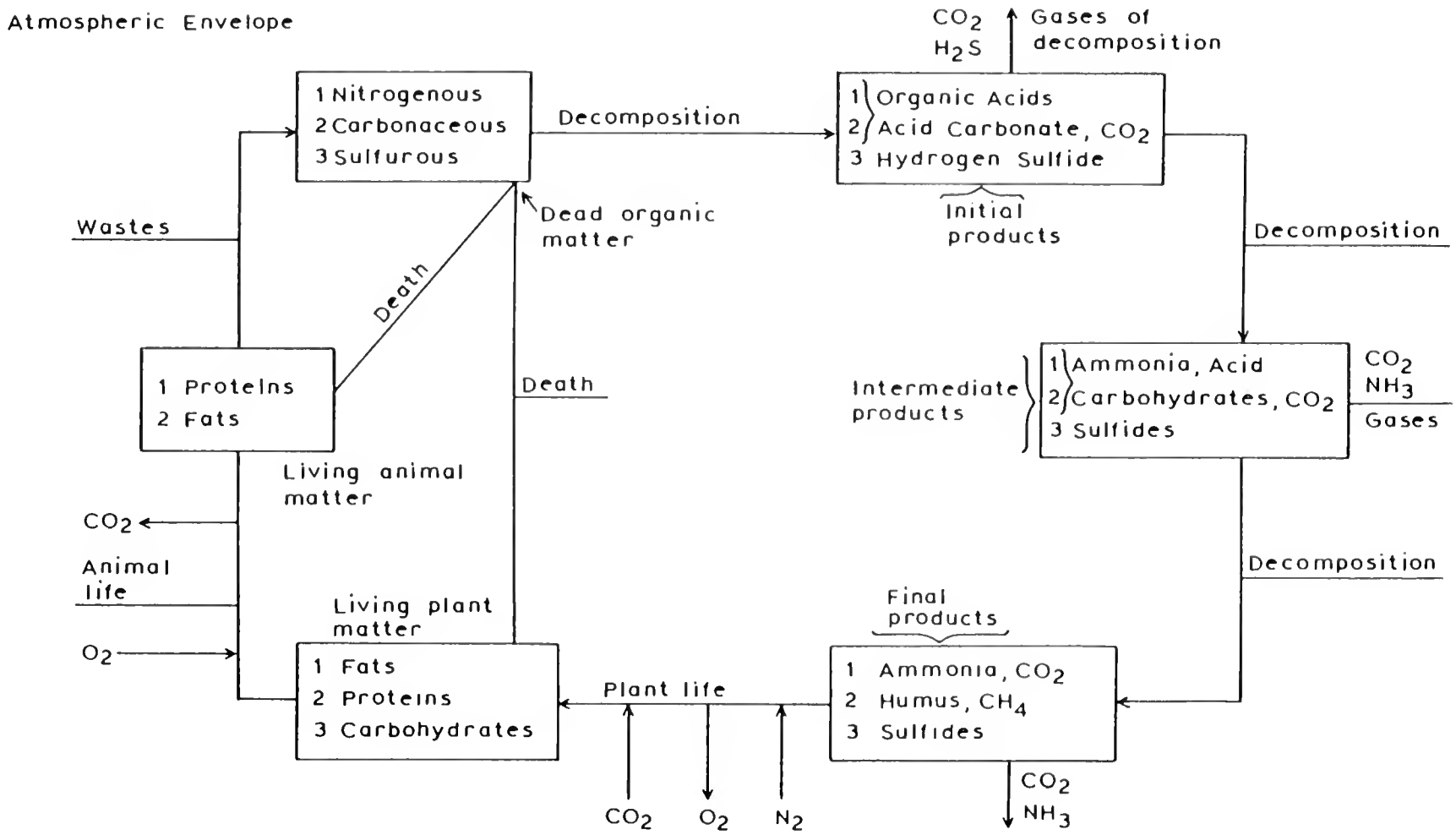

Figure 2-1. The anaerobic cycle in nature (117).

In an anaerobic environment, the various microbiological and biochemical reactions which occur result in an orderly and controlled degradation of the complex organic materials present. Current understanding of the reactions leads to the conceptual development of a staged sequential process (17,19,27), in which organic biodegradation is mediated by the coordinated metabolic activities of groups of facultative and obligate anaerobic bacteria (18,114, 209). The end products of complete anaerobic metabolism are the gaseous compounds methane (CH4) and CO_2, together with a relatively small amount of cell mass (18,114,209). Waste stabilization is directly linked to methane production, the theoretical methane production from 1kg ultimate BOD (BOD_L) or COD being 0.348 m^3 (105). In anaerobic ecosystems exposed to light, bacterial photosynthesis may occur, with resulting primary production of organic matter which becomes an essential part of the microbial food chain (35,42).

2.2 Biochemistry of the Anaerobic Process

In a well-buffered, actively-operating anaerobic system, degradation of complex organics may be conveniently represented by the three-stage biochemical scheme (113,136) illustrated in Figure 2.2 (113,135). The complex organics comprising cellulose, hemicellulose, xylanes, and lignins, together with proteins, lipids and nucleic acids, are hydrolysed, fermented, and mineralized by at least four

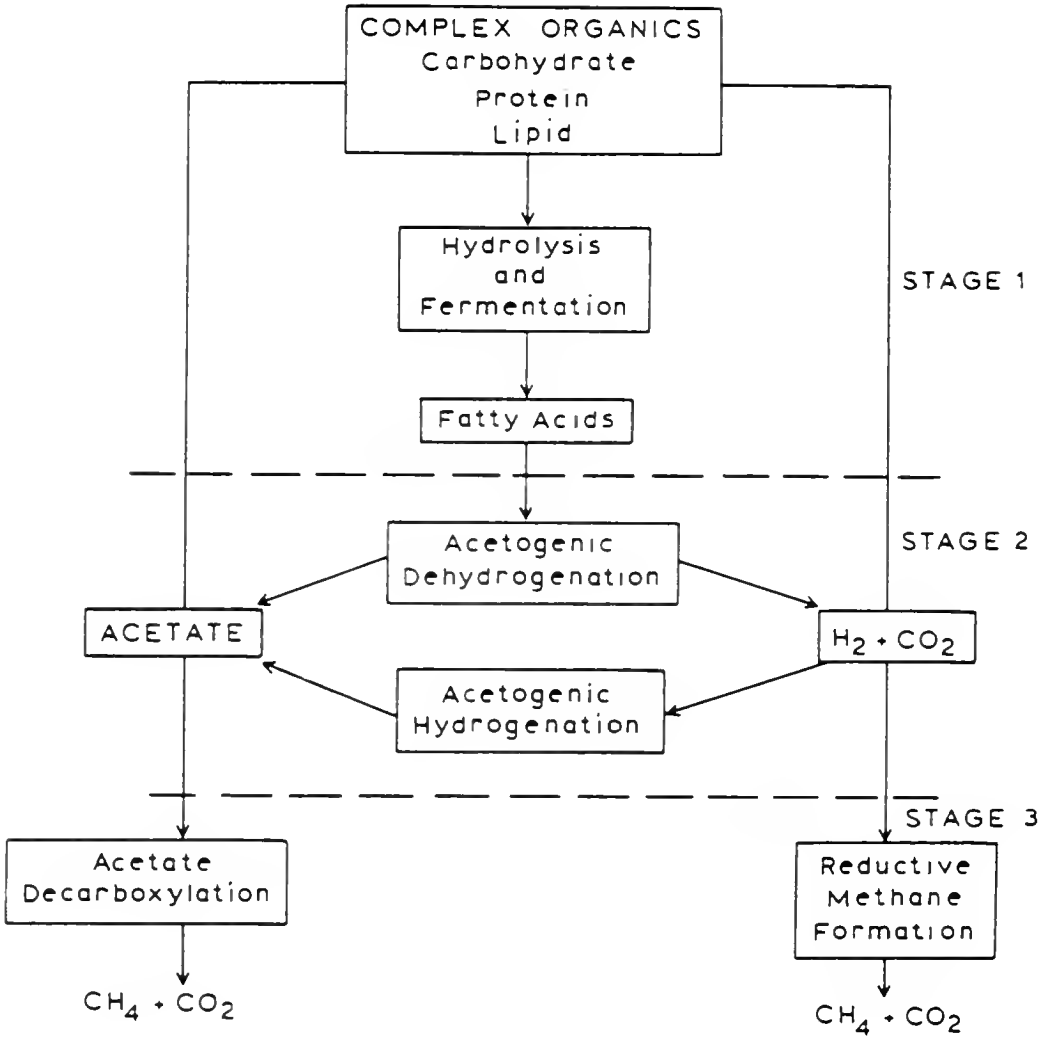

Figure 2-2. Three-stage biochemical scheme for anaerobic biodegradation (113).

different groups of anaerobes, as shown schematically in Figure 2.3 (209), and represented in Table 2-1. First stage enzymatic hydrolysis of complex organics by inducible peripheral enzymes leads to the production of a range of intermediates, which are subsequently used for energy production and growth by the various bacterial species present in the environment. Representative end products of anaerobic microbial degradation of organic wastes are listed in Table 2-2.

Organic polymers such as cellulose, proteins and lipids are first reduced to individual monomers which are then fermented to organic acids, alcohols, CO_2, hydrogen (H_2), acetate, longer chain fatty acids, ammonia (NH_4^+), and sulfide (S^{2-}) (4). The fermented end products are selectively metabolized by the second and third groups of bacteria, which together are responsible for the activities of the second stage. These are the obligate H_2-producing and the homoacetogenic bacteria (209). In this acid-forming stage, the metabolic end products include organic acids, aldehydes, alcohols, mercaptans, and amines. Also produced are H_2, CO_2, H_2S, and ammonia (NH_4^+) (4,19). High molecular weight compounds, such as lignin, are not readily metabolized by anaerobic bacteria. Stabilization of wastes containing cellulose surrounded by polymeric lignin is therefore restricted. Increased anaerobic degradation can, however, be achieved by physico-chemical pretreatment which

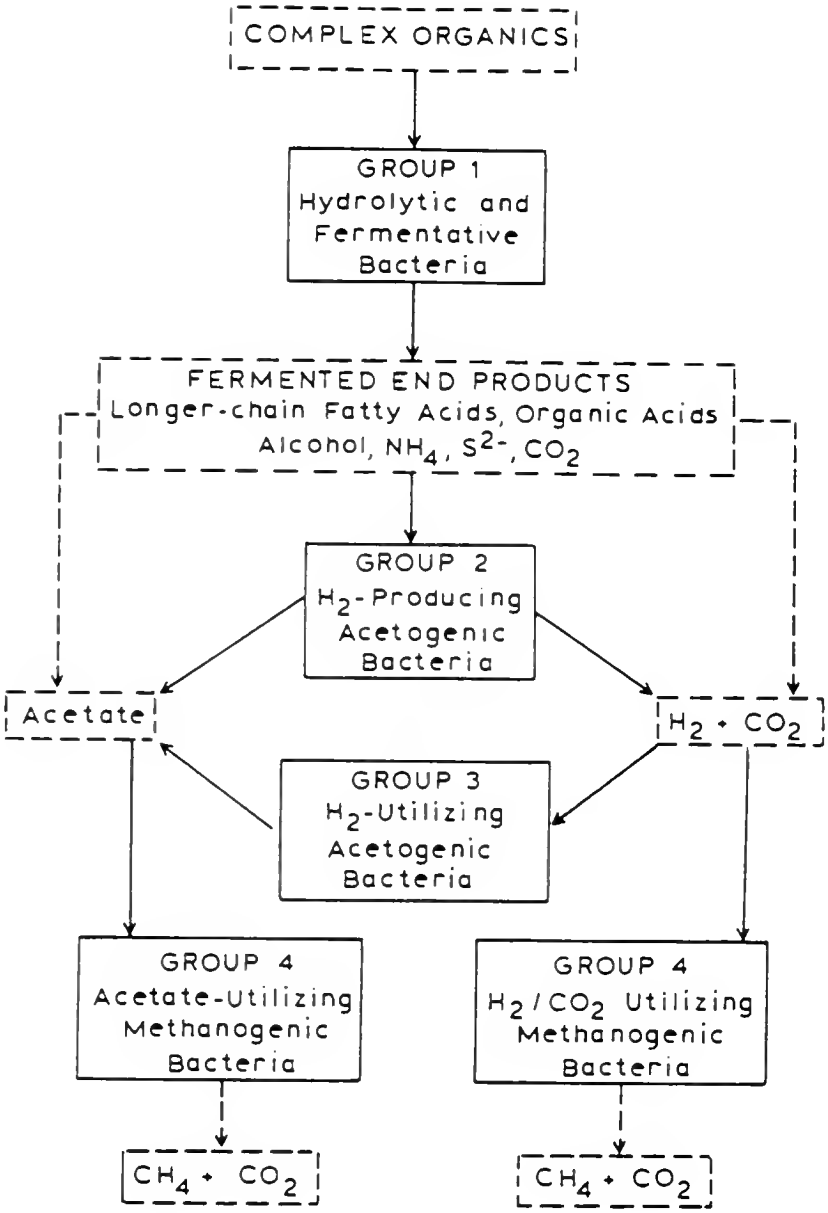

Figure 2-3. Nonphotosynthetic bacterial groups involved in anaerobic biodegradation (200).

Table 2-1. Metabolic patterns of nonphotosynthetic anaerobic bacteria.

Bacterial Metabolic Group	Substrates utilized	Metabolic end products
1. Hydrolytic and fermentative bacteria	Polysaccharides Lipids Proteins	Acetic acid, H_2/CO_2, buyrate, propionate, methanol, ethanol, propanol.
2. Hydrogen-producing acetogenic bacteria.	butyrate, propionate ethanol, propanol	hydrogen, acetate
3. Homoacetogenic bacteria	multi- or one carbon compounds	acetic acid
4. Methanogenic bacteria	acetate, H_2/CO_2, methanol carbon monoxide methylamine	methane, CO_2, H_2O

Source: Zeikus 1980 (209).

Table 2-2. Representative end products of anaerobic microbial degradation of organic wastes.

Substrate	End products
Proteins and other nitrogenous compounds	Amino acids Ammonia Hydrogen sulfide Methane CO_2 H_2 Alcohols Organic acids Phenols Indole
Carbohydrates	CO_2 H_2 Alcohols Fatty acids Neutral compounds
Fats and related substances	Fatty acids Glycerol CO_2 H_2 Alcohols Lower fatty acids
Nucleic acids, purines, pyrimidines	Amino acids Lower fatty acids PO_4 NH_3 CO_2

separates lignin from cellulose or solubilizes the lignin into digestible substrates. The large quantities of ethanol and acetic, formic, and lactic acids produced by acid-forming bacteria become toxic if allowed to accumulate (209), resulting in inhibition of the anaerobic stabilization process. This is prevented by the metabolic activities of terminal trophic groups which transform the metabolites of the first two stages. Principal species are the methanogenic and sulfatereducing bacteria which utilize H_2, one-carbon substrates (formate and methanol), and two-carbon substrates (acetate) as energy sources or electron donors. Phototrophic bacteria may also be included with the terminal organisms in anaerobic environments.

In ecosystems containing low sulfate concentration, the terminal stage of anaerobic degradation is controlled by the activities of methanogenic bacteria (208) which utilize the acetate, H_2 and CO_2 produced in the earlier stages to form methane and carbon dioxide (101,122,123). Estimates vary as to the relative importance of these substrates (162). The interrelationship between methanogenic bacteria and substances of the anaerobic carbon cycle is shown in Figure 2-4 (145), and the approximate percentage distribution of carbon in the metabolic end products of all stages is shown in Figure 2-5.

Single-carbon compounds are readily metabolized in the anaerobic environment. Three principal bacterial groups

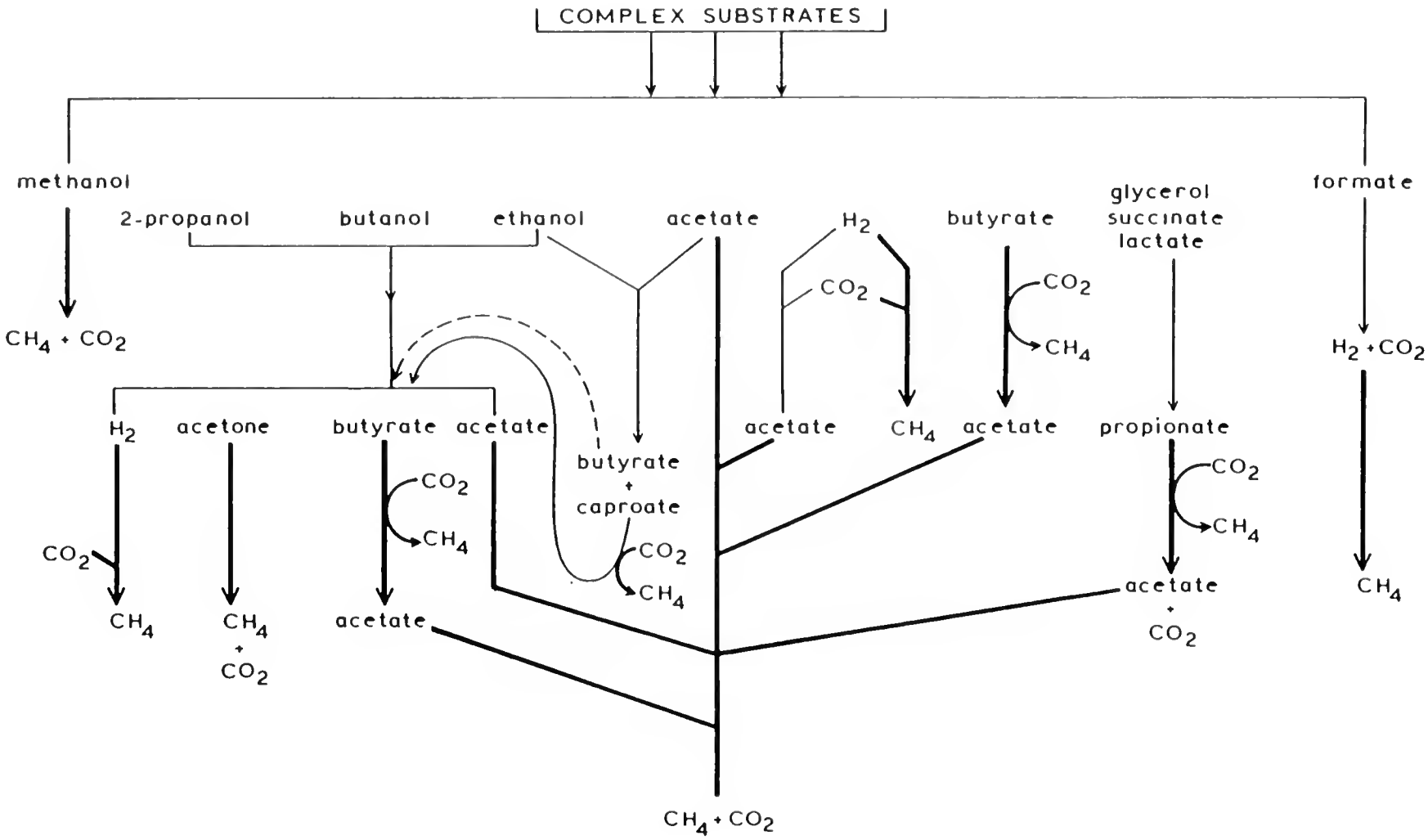

Figure 2-4. Interrelationships between methane bacteria and metabolites of the anaerobic carbon cycle (145).

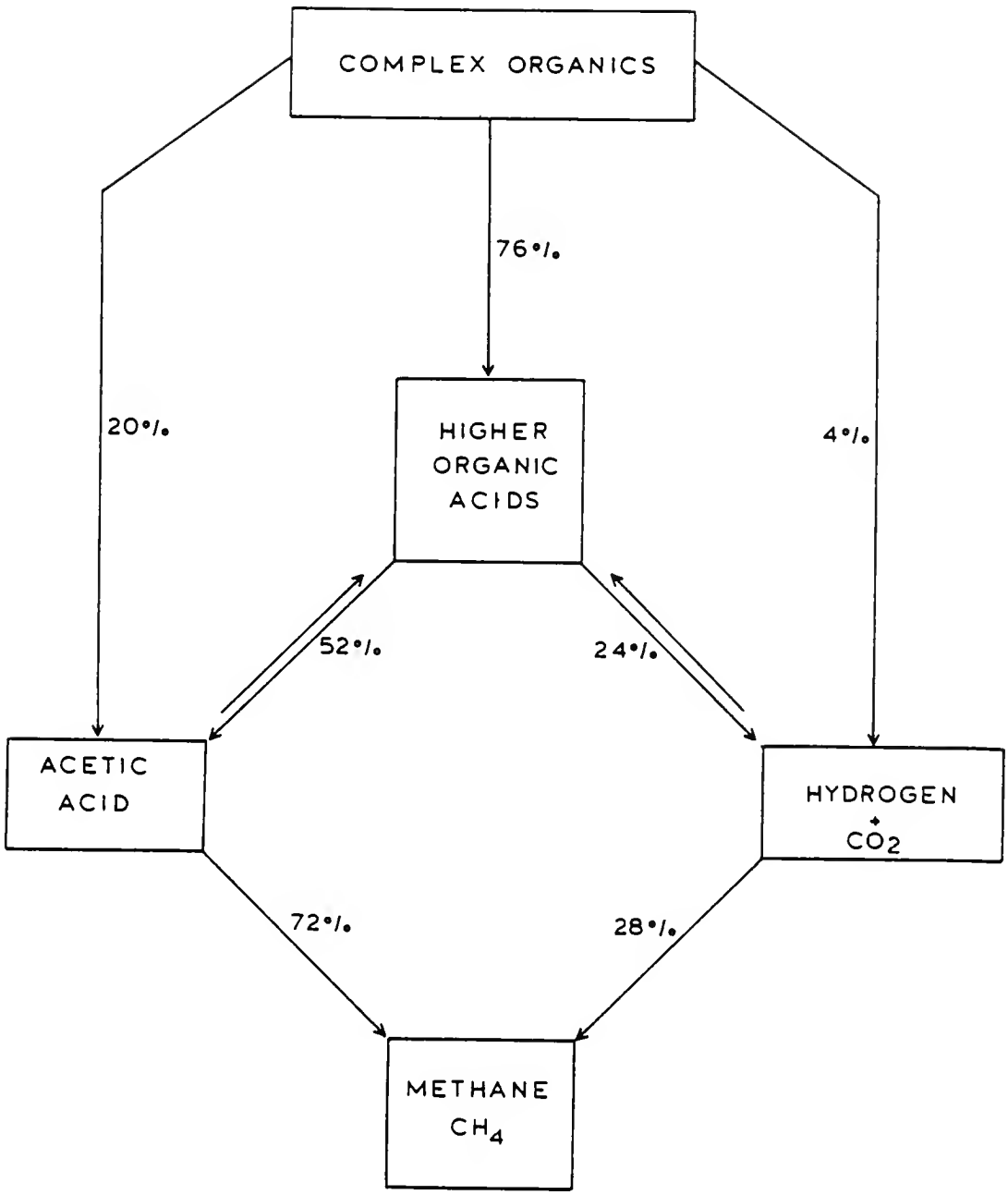

Figure 2-5. Approximate percentage distribution of carbon in metabolic end products of anaerobic biodegradation (162).

consume these substrates as energy sources. These are the methanogens, the sulfate reducers (29) and the homo- or H_2-consuming acetogens. Phototrophic sulfur bacteria utilize H_2 as an electron donor and certain of the other substrates, notably acetate, as carbon source (140).

The sulfate-reducing bacteria will outcompete the methanogens for common energy sources when sulfate is in excess (29), as they have been shown to possess hydrogen metabolism activity with more favorable kinetic properties (lower K_m and higher V_{max}) (149). In such environments, the final stages of conversion of organic molecules into CO_2 will be primarily dependent on these organisms. It has been demonstrated (143) that certain species of the sulfate-reducing bacteria will oxidize long- and short-chain fatty acids and some aromatic compounds to CO_2. For these organisms, elemental sulfur can also act as electron acceptor in place of sulfate (143).

The principal pathway of methane production is dependent on the anaerobic environment. In the rumen, methane is primarily produced through reduction of CO_2 by H_2 (76). In sludge fermentation, most of the methane is formed from acetate (162), although formate is also used by some of these microorganisms. In the latter environment, lipids comprise approximately 28 percent of the organic compounds, and the lipid fraction was found to be primarily responsible for production of acetate from which most of the

methane is formed. This correlates with the high degree of lipid degradation, 65.2 to 90.3 percent, reported by several investigators (34).

In illuminated anaerobic environments, phototrophic anaerobes also play a significant role in the degradative process (139). This they do through the assimilatory metabolism of several of the intermediary metabolites produced by the first three trophic groups above (139). Their metabolic activities also effectively remove H_2S, a potent toxicant, from the environment. Like methanogens, phototrophic bacteria are terminal organisms and, under suitable environmental conditions, will be in direct competition with the former for certain substrates, primarily acetate, H_2 and CO_2.

The trophic divisions and metabolic stages outlined above are not rigidly defined. Certain bacteria bypass the intermediary fermentative stage and metabolize carbohydrates directly to acetate + H_2 in the presence of an H_2-scavenging bacterium (113).

2.3 Microbiology of Anaerobic Environments

2.3.1 Anaerobic Microbial Community

Species composition and methane production within an anaerobic environment are greatly influenced by the characteristics of the organic substrate and environmental factors such as pH, light, temperature and oxygen tension. The

bacterial population of such ecosystems may be conveniently divided into two groups based upon their energy metabolism: (1) nonphotosynthetic anaerobes and (2) photosynthetic anaerobes. The nonphotosynthetic anaerobes include the hydrolytic and fermentative, acetogenic, methanogenic, and sulfate-reducing bacteria. The photosynthetic anaerobes include species of the families Rhodospirillaceae, Chromatiaceae, Chlorobiaceae and Chloroflexaceae.

Identification of the various bacterial species encountered in these environments has been based on the isolation, characterization, and enumeration of predominant microbial populations of bottom muds, anaerobic sludge digesters (103-105), animal manure digesters (74,77), gastrointestinal tracts, and the rumen of cud-chewing animals (77,202). Principal bacterial groups identified are the hydrolytic, acetogenic, methanogenic, phototrophic, and sulfate reducing bacteria (122).

Provided that light can penetrate the anaerobic environment, microorganisms which exist therein are able to achieve an almost completely closed anaerobic cycle of matter, by their ability to metabolize the waste products generated in their ecosystem. Primary synthesis of organic matter under these conditions is mediated by the phototrophic sulfur bacteria which convert CO_2 to cell material using H_2S as reductant (139). Acetate and other simple organic compounds are readily assimilated by phototrophic

nonsulfur bacteria (140), and certain species of phototrophic sulfur bacteria. In some environments, these phototrophic cells are grazed by protozoans. Also, upon death of the phototrophic bacteria, their organic cell components are decomposed by Clostridia and other fermentative anaerobes, with the formation of CO_2, H_2, NH_3, organic acids and alcohols (208). The H_2 and some of the other fermentative products are anaerobically oxidized by sulfate-reducing and methane-producing bacteria.

The sulfide on which the phototrophic sulfur bacteria are dependent for metabolism is produced by the reduction of sulfate and/or the breakdown of proteins into amino acids and subsequent degradation of the amino acids cysteine, cystine and methionine. Proteolytic bacteria responsible for protein degradation include Proteus, Bacteroides spp. and some Clostridium spp. Most of the sulfide is produced by the sulfate-reducing bacterial species Desulfovibrio. Anaerobic oxidation performed by sulfate reducers results in the formation of H_2S and acetate, both utilizable in turn by phototrophic bacteria. The combined activities of sulfate-reducing bacteria and phototrophic bacteria are reflected in the completely closed sulfur cycle illustrated in Figure 2.6. In anaerobic environments, the nitrogen cycle is also closed as the nitrogen atom does not undergo valence changes but alternates between NH_3 and the amino groups ($R\text{-}NH_2$) in nitrogenous cell material.

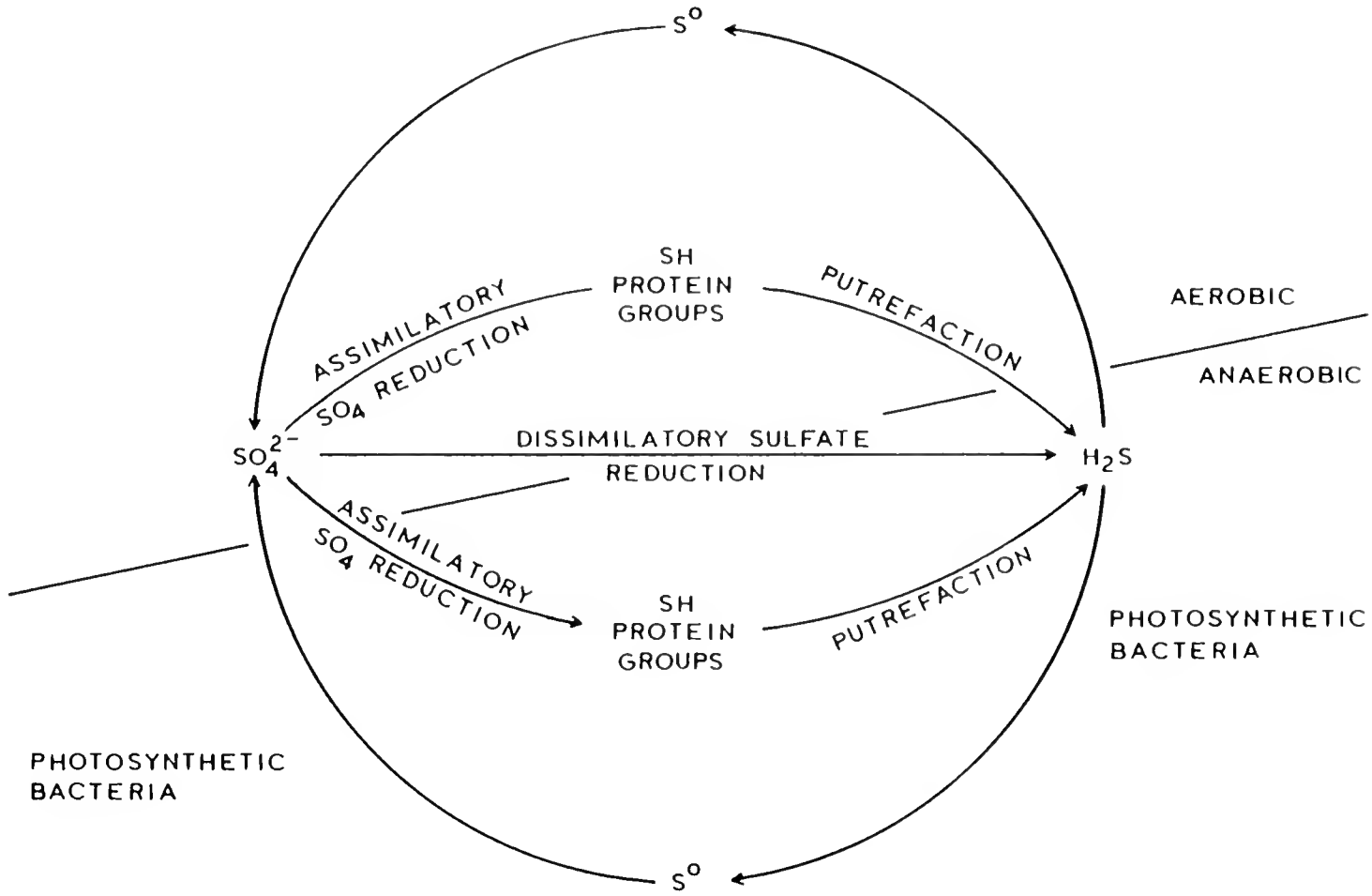

Figure 2-6. The sulfur cycle in nature.

2.3.2 The Nonphototrophic Anaerobes

2.3.2.1. Hydrolytic Bacteria

Hydrolytic bacteria may be Gram-negative or Gram-positive, non-spore-forming or endospore-forming, facultative or obligately anaerobic rods or cocci. They are responsible for initiating the anaerobic degradation of complex organic molecules in the first stage of anaerobic digestion. They produce extracellular or membrane-bound hydrolytic enzymes which hydrolyse polymers of carbohydrates, proteins and, lipids to their soluble monomers which are subsequently fermented to various end products (24). These bacteria are usually coupled to H_2-utilizing bacteria. Representative genera and species which have been identified are listed in Table 2.3 (209).

Distribution of hydrolytic bacteria has been examined by plate counts, and their population has been shown to be highest near the sediment/water interface where the rate of exoenzyme activity correlates with the counts of exoenzyme-producing bacteria (93). The activities of amylase, protease, lipase, and glucosidase in the surface layer of sediment was found to be several orders of magnitude greater than in the water column (81). Bacterial species identified include the proteolytic Clostridium spp., Streptococcus spp. and Eubacterium spp. (19).

Table 2-3. Identification of anaerobic bacterial populations in sewage sludge digesters.

Group	Generic identity and description
Hydrolytic bacteria	Majority unidentified Gram-negative rods; *Clostridium* *Eubacterium*
Hydrogen-producing acetogenic bacteria	Unidentified Gram-negative rods.
Homoacetogenic bacteria	*Acetobacterium* *Clostridium*
Methanogens	*Methanobacterium* *Methanospirillum* *Methanococcus* *Methanosarcina* *Methanothrix*
Sulfate reducers	*Desulfovibrio* *Desulfatomaculum*

Source: Zeikus 1980 (209).

Because of the position of hydrolytic bacteria in the sequentially staged biodegradative process, it has been observed (209) that the rate of methane production in anaerobic digesters is often limited by the rate of biopolymer destruction by these bacteria.

2.3.2.2. The Acetogenic Bacteria

The microbial groups central to anaerobic activity comprise the H_2-producing acetogenic bacteria and the H_2-utilizing (homo-acetogenic) bacteria (209) which convert fatty acids and other compounds to acetate, H_2, and CO_2. In order for them to do this, the hydrogen concentration must be kept very low by the methanogens and other H_2-utilizing organisms (128). Only a few species of H_2-producing acetogenic bacteria have been isolated. This group degrades propionate and longer-chain fatty acids, alcohols, aromatic and other organic acids which are produced in the first stage of fermentation (20).

Indications are that some of these organisms can only be cultured in the presence of hydrogen-metabolizing species (203). Included in this group is the "S organism" isolated from Methanobacillus omelianskii by Bryant and his coworkers (21), which catabolizes ethanol to acetate + H_2. The H_2 is used by M. omelianskii to reduce CO_2 to CH_4 in this syntrophic relationship. Other examples of obligate hydrogen-producing acetogenic bacteria which can metabolize only

in the presence of H_2-scavenging bacteria are Syntrophobacter wolinii (14) which will oxidize propionate to acetate + H_2 only if coupled with a H_2-utilizing organism such as a methanogen or a sulfate-reducing bacterium, and Syntrophomonas wolfei (115) which metabolizes fatty acids of chain lengths up to C_8 by β-oxidation when cocultured with a H_2-utilizing organism. Fatty acids with even numbers of carbon atoms such as butyrate, caproate, and caprylate are oxidized to acetate + H_2 by this bacterium, and those with an odd number of carbon atoms such as valerate and heptanoate are oxidized to acetate + propionate + H_2 (115). Strains of Desulfovibrio desulfuricans and Desulfovibrio vulgaris produce H_2 from lactate or ethanol when grown without sulfate in the presence of H_2-utilizing methanogens (20). Lactate is degraded to acetate in the following manner:

$$CH_2CHOHCOO^- + 2H_2O \text{ --------> } CH_3COO^- + HCO_3^- + H^+ + 2H_2 \qquad (2\text{-}1)$$

Homoacetogenic or hydrogen-consuming acetogenic bacteria have been identified in sewage sludge (15,127). Species of Acetobacterium and Clostridium which are able to metabolize H_2+CO_2, methanol, and/or multicarbon compounds to acetate (6), have been identified. Not all species of Clostridium, however, are capable of metabolizing H_2 (209).

By consuming H_2, these bacteria lower the partial pressure of hydrogen in the anaerobic environment sufficiently for other metabolic activities to be continued.

2.3.2.3. Methanogenic Bacteria

Methanogens are a diverse group of bacteria with considerable variation in nutritional requirements (6). They are strictly anaerobic bacteria which are not able to catabolize alcohols other than methanol or organic acids other than acetate and formate (21). In general, they metabolize one- and two-carbon compounds, utilizing H_2, CO_2, and acetate in the production of CH_4 and CO_2.

Over 12 genera and several dozen species and strains of these bacteria have been described (102). They are broadly classified into two groups, one of which ferments acetic acid to CH_4 and CO_2, while the other produces CH_4 by reducing CO_2, utilizing H_2 or formate (208). The principal users of acetate are the genera Methanosarcina and Methanothrix (161), which produce methane by cleaving the acetate molecule with the formation of CH_4 from the CH_3-group and CO_2 from the carboxyl group.

As illustrated in Figure 2-5, acetate accounts for about 70 percent of the methane produced in digesters or in nature (162). Species of methanogenic bacteria which have been isolated from digesting sludge are listed in Table 2.4 (201). These organisms include the Methanobacterium species

Table 2-4. Methanogenic organisms isolated in pure cultures in digesting sludge.

Organism	Source	Morphology	Gram reaction	Substrates
Methanobacterium ruminantium	rumen and sludge	coccus to short rods in chains	positive	H_2+CO_2 formate
Methanobacterium strain MoH	Methanobacillus omelianskii	irregularly curved rod	variable	H_2 + CO_2
Methanobacterium formicicum	mud and sludge	irregularly curved rod	variable	H_2 + CO_2 formate
Methanosarcina barkerii	mud and sludge	sarcina	positive	H_2 + CO_2 methanol acetate
Methanospirillum sp.	sludge	spirillum	positive	H_2 + CO_2 formate
Methanococcus sp.	sludge	coccus	positive	H_2 + CO_2 formate

Source: Wolfe R.S. 1971 (201)

which have minimal nutrient requirements and grow autotrophically on H_2+CO_2 with sulfide and ammonia as sole sources of sulfur and nitrogen, respectively.

Methanogens are terminal organisms in the sequential anaerobic degradative chain. They are nutritionally simple (104,208) and utilize potentially toxic compounds produced from anaerobic fermentation or respiration of organic material. Most of these bacteria use H_2 as an energy source in reducing CO_2 to CH_4 and influence the carbon and electron flow in anaerobic habitats by an interaction termed interspecies hydrogen transfer. Compounds utilized by methanogenic bacteria as energy sources for methane production are listed in Table 2.5. Methanogens are primarily autotrophic, but the rumen species Methanobacterium ruminantium and Methanobacterium mobile require various fatty acids, amino acids, co-factors and B-vitamins for growth (104,208).

All species, except Methanobacterium arbophilicum, Methanobacterium thermoautrophicum, and Methanobacterium barkeri, in addition to utilizing H_2 in reducing CO_2 to CH_4, will also utilize formate as an energy source for this purpose (104,208). Methanosarcina barkeri is the only known species which will form methane from both methanol and acetate (104,208). Ammonia is generally used as the nitrogen source, phosphate as the source of phosphorus, and sulfide or cysteine as the source of sulfur (102).

Table 2-5. Compounds utilized by Methanogenic bacteria as energy sources for methane production.

Compound	Methanogenic species
H_2/CO_2 or Formate/CO_2	Methanobacterium formicicum Methanobacterium thermoautotrophicum Methanobacterium ruminantium Methanobacterium mobile Methanosarcina barkeri Methanococcus vanniellii
Acetate	Methanobacterium soehngenii Methanosarcina methanica Methanosarcina barkeri Methanococcus mazei
Butyrate	Methanosarcina methanica
Methanol	Methanosarcina barkeri
CO	Methanosarcina barkeri

Source: Mah and Smith 1981 (102).

Methanogens are extremely sensitive to environmental factors such as oxygen (O_2) and require a highly reduced environment for growth, the required oxidation/reduction potential (E_c) being in the range -520 to -530 mV (43). For swine waste studied under minimal aeration, it was found that methane production ceased at E_c of -360 mV. The required pH range for methane production is 6.6-7.6 with an optimum of 7.0-7.2, but acid production will continue to pH 4.5 (106). At pH > 7.4, ammonia in the form of NH_3 is considered inhibitory at concentrations of 1500-3000 mg/L, and above 3000 mg/L the ammonium ion becomes toxic regardless of pH (93,107). It has, however, been reported (37) that under certain conditions, methane may be produced at ammonia concentrations in excess of 3000 mg/L.

Two optimum levels of temperature have been established for methane production, 35-40° C in the mesophilic range, and 55-60° C in the thermophilic range. Sulfides can be extremely toxic, but concentrations of up to 200 mg/L may be tolerated by methanogens with some acclimation (95).

Operational stability of a methane-producing system is also largely dependent on the buffering of the system. The bicarbonate buffering capacity of a system will be influenced by the protein content of the substrate as well as the amount of CO_2 produced. Biodegradable protein is deaminated to produce ammonia which reacts with water as follows:

$$NH_3 + HOH \text{ ------> } NH^{4+} + OH- \quad (2\text{-}2)$$

The OH- reacts with CO_2 in water to form bicarbonate ions

$$CO_2 + HOH \text{ ----> } H_2CO_3 \text{ ---> } H^+ + HCO_3 \quad (2\text{-}3)$$

$$H_2CO_3 + OH- \text{ ----> } HCO_3- + HOH \quad (2\text{-}4)$$

2.3.3 The Phototrophic Anaerobes

2.3.3.1 General Description

Phototrophic anaerobes are a physiologically diverse group of Gram-negative aquatic bacteria which perform an anoxygenic type of photosynthesis under anaerobic conditions, using only one photosystem (142). These anaerobes, which may be broadly divided into two groups, the phototrophic sulfur bacteria, and the phototrophic nonsulfur bacteria, contain photosynthetic pigments of the bacteriochlorophyll type, and typical carotenoid pigments (139). Phototrophic sulfur bacteria are obligate anaerobes which are dependent upon the presence of oxidizable external electron donors such as reduced sulfur compounds, molecular hydrogen, or organic carbon compounds, primarily acetate, for their metabolism (138). Assimilatory sulfate reduction is lacking in these organisms and sulfide is required as a source of reduced sulfur for biosynthesis (140). All

species contain cytochromes, ubiquinones, and nonheme iron proteins as components of their electron transport systems (140).

2.3.3.2 Classification

In the classification of phototrophic bacteria, Order Rhodospirillales is divided into two sub-orders, Rhodospirillineae, and Chlorobiineae (141).

Suborder Rhodospirillineae is characterized by those bacteria which contain bacteriochlorophyll a or b as the major bacteriochlorophyll, and carry their photopigments in intracytoplasmic membrane systems continuous with the cytoplasmic membrane. This suborder contains the two families Rhodospirillaceae (purple nonsulfur bacteria), and Chromatiaceae (purple sulfur bacteria).

Rhodospirillaceae are facultative heterotrophs, in addition to being phototrophs. They do not grow well in a sulfur or sulfide-containing environment and are generally unable to use hydrogen sulfide (188). The single exception is Rhodopseudomonas sulfidophila which grows well with sulfide (61). They are unicellular, Gram-negative, straight-, curved-, or helical-rods which are usually non-gas-vacuolated. They have well defined guanine plus cytosine (G + C) ratios ranging from 61-70 percent and are flagellated when motile (10,178). This family primarily uses

simple organic compounds such as alcohols and acids which act as electron donors and are photoassimilated (140).

All species of the family Chromatiaceae are capable of photolithotrophic CO_2 fixation in the presence of sulfide and sulfur (9) during which, with the exception of the genus Ectothiorhodospira, sulfur is deposited inside the cells (178). Molecular hydrogen is also used as electron donor by many species of this family. Some species will photoassimilate acetate and pyruvate (139). These bacteria are Gram-negative, frequently gas-vacuolated, spherical, ovoid, rod-, vibrio-, or spiral-shaped cells which display heterogeneous guanosine + cytosine (G + C) ratios ranging from 45-70.4 percent.

The family Chromatiaceae comprises two main physiological-ecological groups representing ten genera and 26 species of bacteria (140). One group which includes Amoebobacter, Lamprocystis, Thiodictyon, and Thiopedia possesses gas vacuoles, thus enabling them to migrate vertically within the water column (139). The second group, which includes small Chromatium, Thiocystis, Thiocapsa, Thiosarcina, and Ectothiorhodospira does not possess gas vacuoles. All of these bacteria are able to develop either in single cell or nonmotile aggregates aggregates of cells. Both forms are features of the purple-red blooms of Chromatiaceae observed in lagoons, shallow pools and estuarine environments (139).

Suborder Chlorobiineae also contains two families, the Chlorobiaceae (green sulfur bacteria) and the Chloroflexaceae (gliding bacteria). In these families, the major bacteriochlorophylls are c, d, or e along with small amounts of bchl a in the photosynthetic reaction centers. In addition, the green-colored species of this family possess the carotenoids chlorobactene and OH-chlorobactene, whereas the brown-colored species possess the carotenoids isorenieratene and -isorenieratene (98) which contribute to their color and the broader absorption range between 480 and 550 nm. The in vivo long wavelength absorption maxima of the major bacteriochlorophylls (bchl) are: bchl a 830-890 nm, bchl b 835-850 and 1020-1040 nm, bchl c 745-755 nm, bchl d 705-740 nm, and bchl e 719-726 nm. Suborder Chlorobiineae is also characterized by the chlorobium vesicles which contain the photosynthetic apparatus and occur as special organelles underlying and firmly fixed to the cytoplasmic membrane (138). Chlorobiaceae, with the exception of the genus Chloropseudomonas, are nonmotile, frequently gas-vacuolated, spherical, ovoid- or rod-shaped cells (140). They are obligately anaerobic organisms which utilize sulfide as an electron donor, deposit elemental sulfur extracellularly, and are incapable of assimilatory sulfate reduction (178). Their G + C ratios are well defined, ranging from 48.5-58.1 percent (142). They metabolize certain organic compounds, notably acetate and propionate (138,139).

Chloroflexaceae (175) are Gram-negative, filamentous, gliding, anoxygenic phototrophs with flexible cell walls. Of this family, only one species, Chloroflexus aurantiacus has been studied in pure culture (144). These bacteria exhibit anoxygenic photosynthesis using reduced sulfur compounds as electron donors, but their best growth occurs in the light when using fixed carbon compounds.

2.3.3.3 Photometabolism

During autotrophic growth, photosynthetic fixation of CO_2 by the Chromatiaceae is primarily by the reductive pentose phosphate cycle (139). This cycle is of limited importance to Chlorobiaceae which utilize a cycle of reactions involving ferredoxin-dependent carboxylations, catalyzed by pyruvate- and a-ketoglutarate synthase (22). Other reactions involving carboxylic acid enzymes, have also been shown to be of significance (23) for this family. In the green sulfur bacteria a mechanism of CO_2 fixation involving a reverse tricarboxylic acid (TCA) cycle has been proposed (49,154,156) and accepted as the major route of carbon fixation (53).

This cycle, which is illustrated in Figure 2-7, involves two ferredoxin-dependent carboxylations. One complete turn of the cycle produces a molecule of oxaloacetate from four molecules of CO_2 (155). Intermediates of the cycle include precursors for lipids and amino

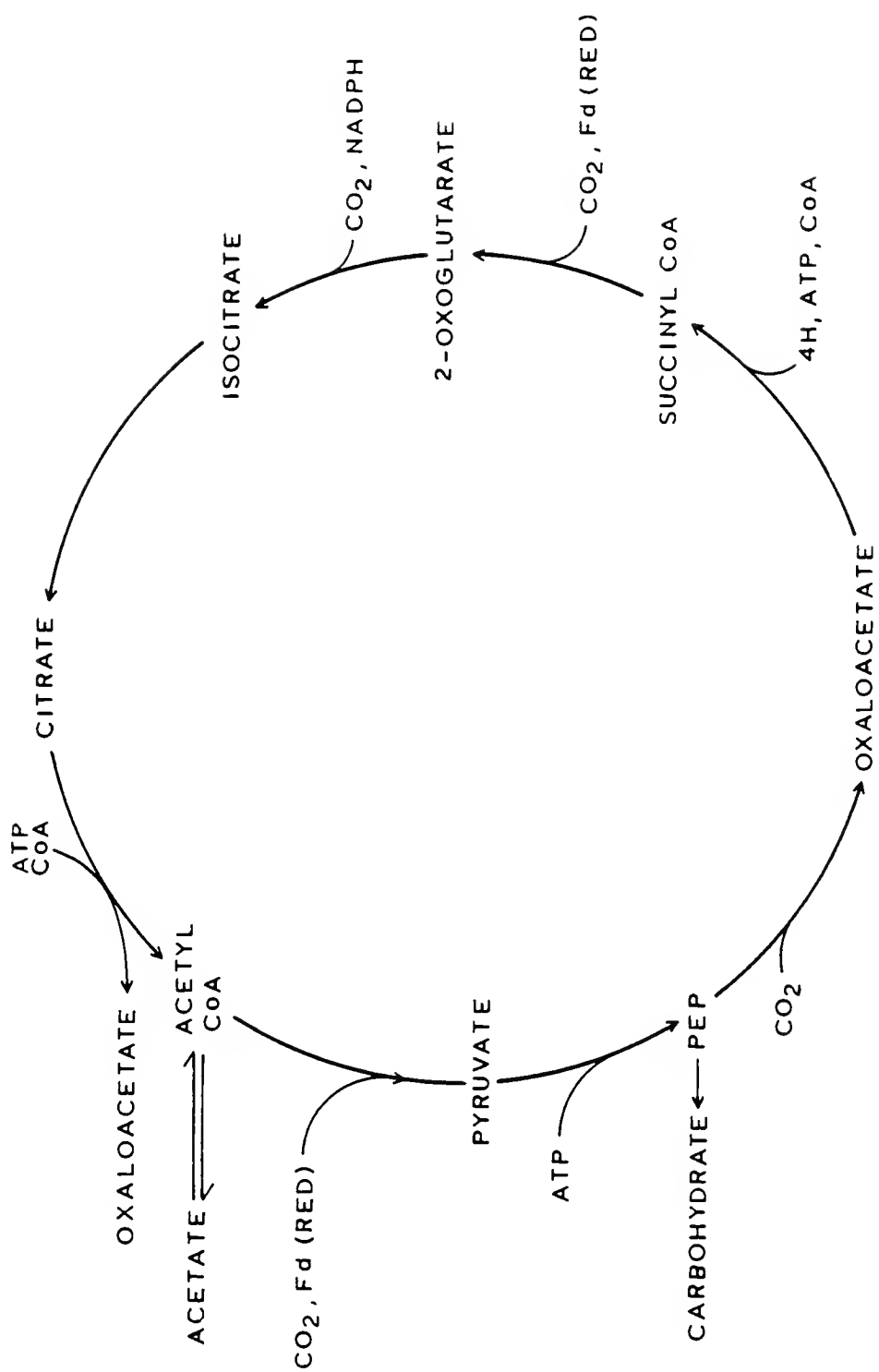

Figure 2-7. The reductive tricarboxylic acid cycle of green sulfur bacteria.

acids; carbohydrate is formed from pyruvate by reversed glycolysis. Species of the family Rhodospirillaceae possess a complete TCA cycle which functions oxidatively in the dark as well as anaerobically in the light (54).

Primary contribution to current understanding of the biochemistry of photosynthetic CO_2 fixation has been made by Van Niel (190) who postulated the unitary concept of photosynthesis

$$CO_2 + 2H_2A \text{ ----------> } CH_2O + H_2O + 2A \qquad (2\text{-}5)$$

where H_2A may represent either water, as in the case of green plant photosynthesis, or hydrogen sulfide, as in bacterial photosynthesis. Cell mass is represented by CH_2O.

Detailed studies on the photoassimilation of carbon have been carried out in only a few of the more than 50 known species of phototrophic bacteria. The role of compounds metabolized by phototrophic bacteria is indicated in Table 2.6 (140), and some of the known organic compounds metabolized by these bacteria are listed in Table 2.7 (41). The photometabolic activity of sulfur bacteria is dependent on the presence of oxidizable external electron donors such as reduced sulfur compounds (139). The amount of sulfide removed from the environment by these organisms during metabolic activity is dependent on the carbon source being

Table 2-6. Role of compounds metabolized by the phototrophic bacteria

Substrate	Bacteria	Metabolic role
H_2 H_2S $Na_2S_2O_3$ S	S, NS S	H donor H donor
CH_4	NS	C source, H donor
N_2	S, NS	C source
N_2	S, NS	N source
NH_3	S, NS	N source
Simple organic acids	S, NS	
Amino acids	S, NS	
Peptones	S, NS	
Fats, oils	NS	growth substrates
Sugars	NS	
Alcohols	NS	
Ketones	NS	
Aromatics	NS	

S, sulfur bacteria (Chromatiaceae, Chlorobiaceae)
NS, nonsulfur bacteria (Rhodospirillaceae)
Source: Crofts A.R. 1971 (41).

Table 2-7. Some organic compounds photoassimilated by phototrophic bacteria.

Compound	Bacterial Genera and Species
Acetate	Chromatium, Chloropseudomonas, Thiocystis Lamprocystis, Thiospirillum, Thiocapsa, Amoebobacter, Ectothiorhodospira,
Pyruvate	Chromatium, Thiocystis, Thiocapsa, Lamprocystis, Thiodictyon, Chlorobium, Amoebobacter, Ectothiorhodospira, Chloropseudomonas.
Glucose	Chromatium, Amoebobacter, Ectothiorhodospira, Chloropseudomonas, Thiocystis.
Intermediates of TCA Cycle	Chromatium vinosum
Fructose	Thiocapsa roseopersicina, Amoebobacter, Ectothiorhodospira, Chlorobium, Thiocystis
Fumarate	Thiocapsa roseopersicina
Glycerol	Thiocapsa roseopersicina, Chloropseudomonas
Malate	Thiocapsa roseopersicina, Amoebobacter, Ectothiorhodospira
Succinate	Thiocapsa roseopersicina
Propionate	Thiocapsa pfenigii, Ectothiorhodospira, Chlorobvium, Pelodictyon.
Amino acids	Amoebobacter
Butyrate	Ectothiorhodospira
Lactate	Ectothiorhodospira, Chloropseudomonas
Glutamate	Chlorobium
Peptone	Chlorobium
Ethanol	Chloropseudomonas
Formate	Chloropseudomonas

Source: Mah and Smith 1981 (102).

utilized (185). Sulfide uptake during the metabolism of CO_2 is indicated in the following reaction:

$$40CO_2 + 21H_2S + 8NH_3 + 20H_2O \longrightarrow 8(C_5H_8O_2N) + 21H_2SO_4 \qquad (2\text{-}6)$$

and for acetate

$$40C_2H_4O_2 + H_2S + 8NH_3 \longrightarrow 8(C_5H_8O_2N) + H_2SO_4 + 20H_2O \qquad (2\text{-}7)$$

From these equations it may be observed that sulfide uptake by phototrophic sulfur bacteria is 21 times greater during CO_2 metabolism than during acetate metabolism (185).

The key enzymes involved in photoassimilation of CO_2 via the reductive pentose cycle are ribulose 1, 5-diphosphate carboxylase and ribulose 5-phosphate kinase (142). Enzymes of the Calvin cycle are repressed in certain species of the Chromatiaceae when they are grown anaerobically in light with acetate as the sole carbon source. At such times, metabolism is via the glyoxalate cycle. Green sulfur bacteria are very sensitive to oxygen, but purple sulfur bacteria are not killed by oxygen. In certain environments, some species of purple sulfur bacteria are able to grow chemoautotrophically in the dark, oxidizing H_2S with O_2 (82). Ribulose bisphosphate carboxylase of the latter organisms possesses oxygenase activity, and it has been

demonstrated (170) that the cells excrete glycolate in the presence of O_2.

Certain simple organic substrates are photoassimilated by Chlorobiaceae in the presence of sulfide. These include acetate, propionate, butyrate, lactate and some amino acids (83). The amount of acetate assimilated is directly proportional to either the sulfide or the bicarbonate concentration when either is growth limiting.

2.3.3.4 Energetics

Microbial growth results from coordinated synthesis of a range of complex macromolecules utilizing an energy source appropriate to the particular organism. During biological oxidations, the energy present in an organic substrate is released by successive dehydrogenations of the carbon chain. Reducing equivalents are removed in pairs and transferred to a final acceptor which may be O_2, in the case of aerobic respiration, inorganic compounds other than O_2 in the case of anaerobic respiration, or organic compounds in the case of fermentation. Transfer proceeds via electron transport systems.

Approximately 80 percent of a cell's energy budget is expended on biosynthetic processes, as indicated in Table 2.8 (167). In chemotrophic organisms, this energy is obtained from nutrients in their environment. In phototrophs an energy source external to their environment,

Table 2-8. Bacterial energy budget for cells grown on glucose.

Process	Percent Energy (ATP) expended on each process.
Synthesis	
Polysaccharide	6.5
Protein	61.1
Lipid	0.4
Nucleic Acid	13.5
Transport into cells	18.3

Source: Stouthamer 1973(167).

in the form of light of suitable wavelength, is required. About 60 percent of the biosynthetic energy requirement is utilized for protein synthesis, and nutrient transport accounts for about 18 percent (167).

In all cells, the main energy-coupling agent is adenosine triphosphate (ATP) (97), which is generated by the reaction

$$\underset{\text{Adenosine diphosphate}}{ADP} + \underset{\text{inorganic phosphate}}{P_i} \text{ -----> } \underset{\text{Adenosine triphosphate}}{ATP} + H_2O \qquad (2\text{-}8)$$

When hydrolyzed, ATP yields a standard free energy change $\Delta G_o'$ of -7.0 kcal.mol^{-1}. This energy drives solute transport across the cytoplasmic membrane. Reactions with a $\Delta G_o'$ less than -7.0 kcal.mol^{-1} cannot be coupled directly to ATP generation (163).

ATP synthesis in bacteria occurs either by substrate level phosphorylation or by chemiosmotic energy-generating processes (172), commonly referred to as electron transport (oxidative) phosphorylation. In substrate level phosphorylation, in which a phosphate molocule is first added to a substrate, and is then subsequently transferred to ADP to form ATP, one molecule of ATP is synthesized by phosphorylation of one molecule of substrate in the cell (97). Oxidative phosphorylation, which had been hypothesized for some time (119,120) but only recently has been demonstrated

experimentally (158), provides no direct generation of ATP. Instead, an electrochemical proton gradient is developed (119, 120), and electrogenic proton pumps translocate protons across the cytoplasmic membrane from the cytoplasm to the external medium; this generates an electrical potential and a pH gradient across the membrane.

These energy-transducing systems in the membrane convert chemical energy or light energy into electrochemical energy which is used to drive energy-requiring processes (158). In anaerobic environments, fermentations are carried out by a variety of heterotrophic bacteria. These result in the generation of ATP by substrate level phosphorylation, and formation of several intermediate stage metabolic end products. Photoautotrophs derive their energy from an external source not utilized by heterotrophs with which they share the habitat (140). They are able to fix CO_2, a major waste product of their cohabitants, using H_2S, another waste product as electron donor.

Phototrophic sulfur bacteria obtain their energy from light, transform it via cytochromes, and finally store it as ATP (139). Unlike eucaryotic phototrophs and the cyanobacteria, these organisms carry out an anoxygenic photosynthesis using only one photosystem. They therefore require electron donors of lower redox potential than water, and generally utilize reduced sulfur compounds, molecular hydrogen, or simple organic compounds for this purpose.

These compounds are either present in the environment or are produced by metabolic activities of other organisms (139). Under anaerobic conditions in the dark, phototrophic bacteria obtain their energy for maintenance by fermentation of storage polysaccharides (11).

A comparative illustration of the two types of photosystems is given in Figure 2-8 (50). In bacterial photosynthesis, the electron absorbed by the reaction center chlorophyll P870 is raised to an acceptor designated X (186). It then passes down through an electron transport system involving ubiquinones, generating ATP during this cycle. In the oxygenic system, ATP is generated by cyclic photophosphorylation in Photosystem I, and NAD(P)H is also produced by this photosystem. ATP is generated in Photosystem 2 by noncyclic photophosphorylation, this being the normal process in green plants. When NAD(P)H is produced in Photosystem I, the electrons diverted to $NAD(P)^+$ are replaced from Photosystem 2. In anoxygenic photosynthesis, NAD(P)H cannot be produced directly by Photosystem I in most instances.

In green sulfur bacteria, the redox potential of X is apparently lower than that of the purple sulfur bacteria, enabling the former to reduce ferredoxin (Fd) and NAD(P) directly (128). Purple bacteria apparently are unable to reduce NAD(P) directly by the photosystem. In such cases NAD(P) reduction occurs either by expending ATP (Figure

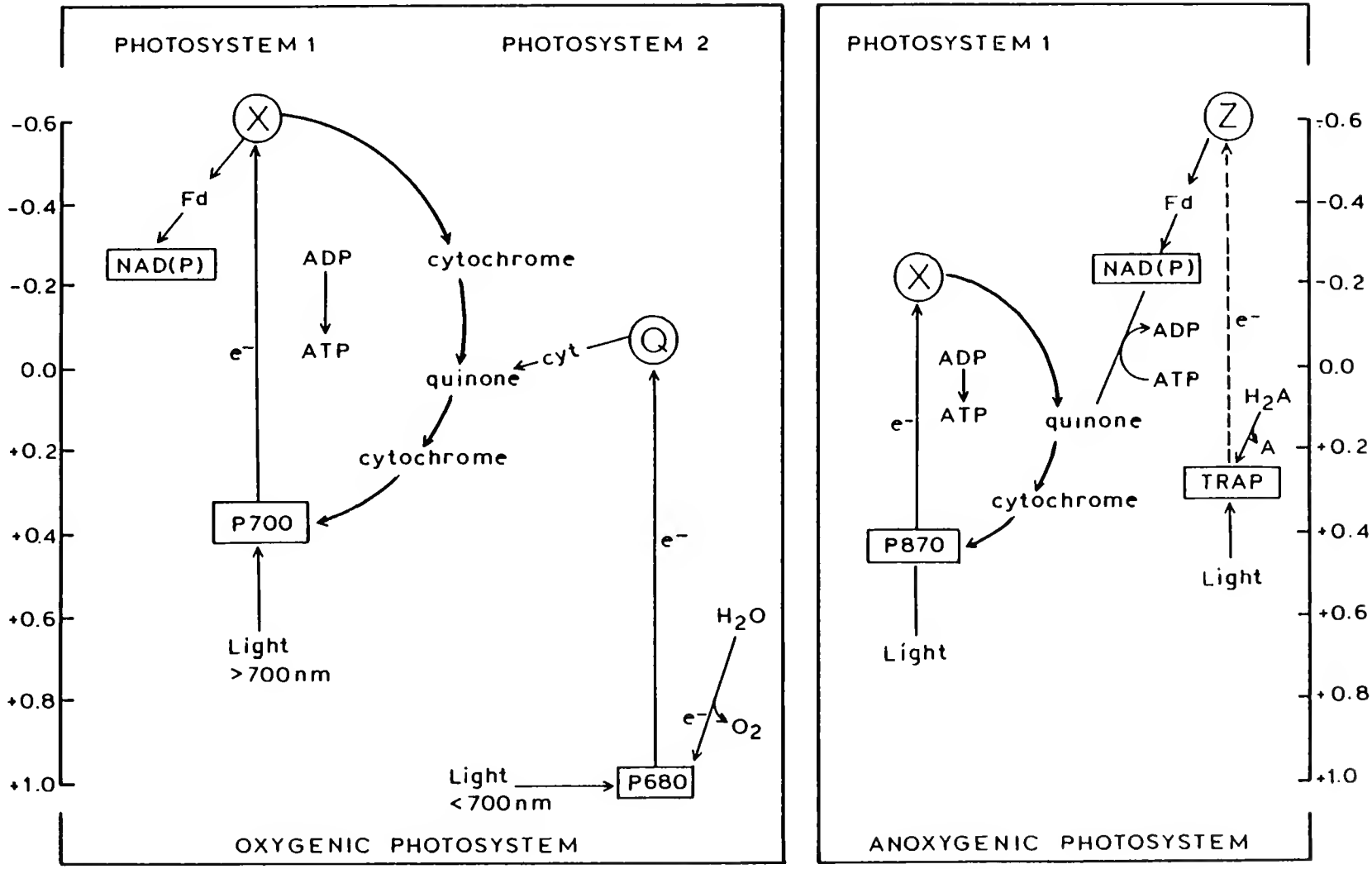

Figure 2-8. Simplified comparative illustrations of oxygenic and anoxygenic photosystems (50).

2-8) or by a process of reverse electron transfer effected by an external electron donor (5). This is shown in Figure 2-9.

By utilizing an external energy source, phototrophs are able to make opportunistic use of any organic matter available to them in their environment. The presence of a functional TCA cycle indicates a potential for acetate metabolism (139).

The electron donors utilized by these organisms are oxidized by different enzyme complexes and pathways. Thus, H_2 reduces NAD^+, and the resulting NADH is oxidized via the electron transport chain. In some phototrophic bacteria, reduced sulfur compounds release electrons upon oxidation at potentials too high to reduce NAD^+, hence NADH for biosynthesis must be provided via ATP-dependent reversed electron transport (139).

The role of ATP in photometabolism is two-fold. It is required either in forming an "activated" substrate, to bring CO_2 fixation into the Calvin cycle, or an "activated" carbon source such as acetyl-CoA from acetal and co-enzyme A (CoA) (48). The activated compounds are then involved in synthetic reactions, which are catalyzed by specific enzyme systems which function in the dark.

Phototrophic bacteria differ amongst themselves in the nature of their light-harvesting or antenna pigments. The electron transport systems and the light harvesting pigments

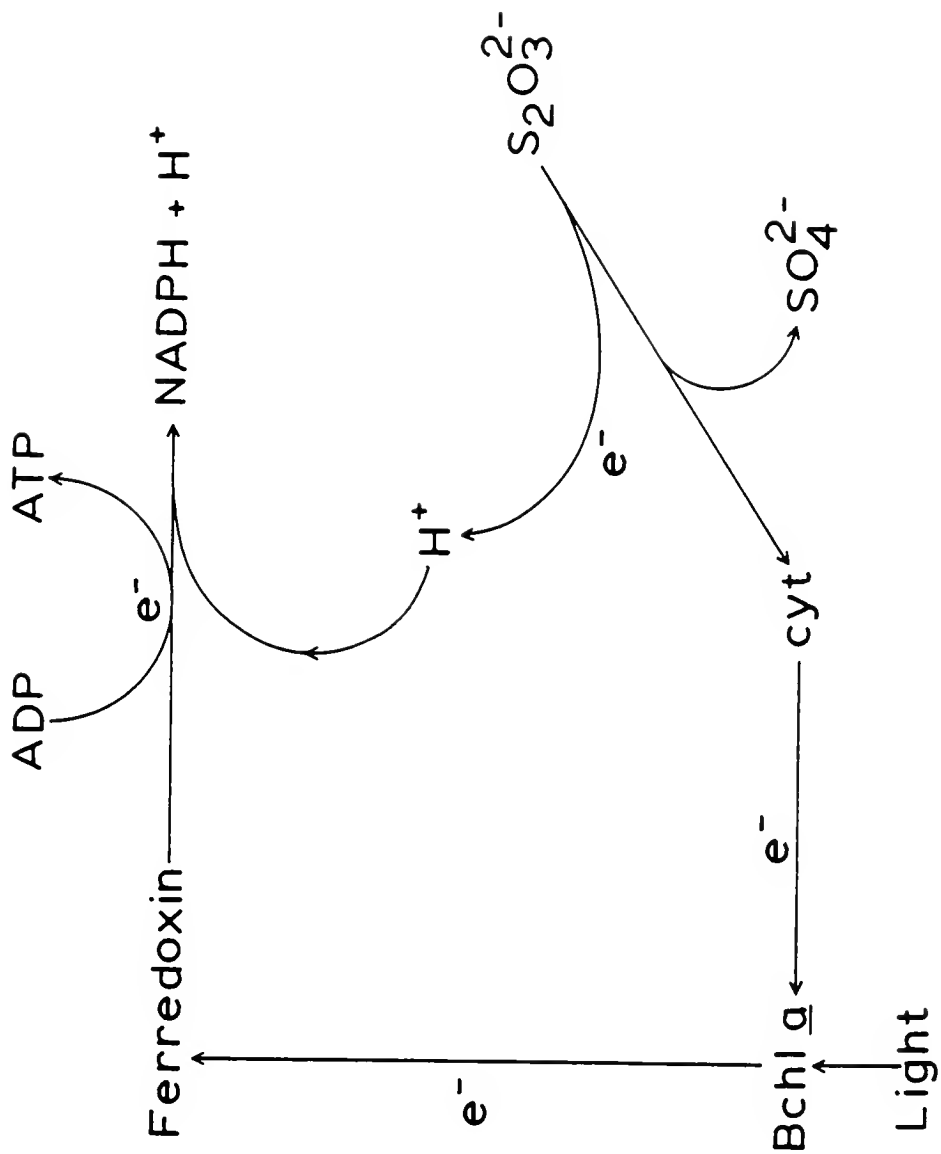

Figure 2-9. Scheme for photosynthetic NAD(P) reduction in purple sulfur bacteria (5).

are associated with chromatophores, the small vesicles formed by the invaginated intracytoplasmic membrane (133). Light energy harvested by antenna pigments migrates to the bacterial reaction center, which contains bacteriochlorophyll a, and where electron transfer reactions occur (133). In these bacteria, ATP generation by cyclic photophosphorylation is not directly linked to substrate utilization, as is the case with heterotrophic bacteria.

2.3.3.5 Ecology of Phototrophic Bacteria

Phototrophic bacteria are restricted in ecological distribution by their need for anaerobic conditions in the light (139). Their presence has been catalogued in a wide range of aquatic habitats including lakes, ponds, sewage ponds and lagoons treating other high strength wastes, salt and freshwater pools, mud flats, sulfur springs, and paddy soils. They have been found in environments with salt content ranging from near zero to saturation and at temperatures ranging from below zero to about 80° C (26). The existence of purple and green bacteria in these habitats is generally indicated by the pink and green blooms, which are observed especially below the thermocline in lakes (176).

Shallow ponds, mud flats, and similar locations which are rich in organic matter, CO_2, H_2, and H_2S, are ideal habitats for phototrophic bacteria that photometabolize all

end products of fermentative reactions (139). Except near the air/water interface, such environments are free of oxygen, and phototrophic bacteria can grow near the water surface where light intensity is high. Here the ability of phototrophic anaerobes to absorb light of very long wavelength is critical to their survival. Such far red and infrared light is transmitted by the overlying phototrophs and is absorbed by the bacteria.

In the photic zones of freshwater lakes and seas, the dominant organisms are primary producers. The optical properties of such bodies of water are important regulatory parameters in the physiology and behaviour of organisms which reside therein. Very dense populations of phototrophic bacteria have been found at various depths in a number of meromictic lakes (132,171,176), which are characterized by permanent stratification of the water. In such lakes, the aerobic upper layer is underlain by a cold oxygen-free zone in which anoxygenic phototrophs exist, normally in a narrow band just within the anaerobic layer (187). At this depth, the overlying water column becomes an effective light filter, transmitting only green and blue-green light of wavelengths between 450 and 550 nm. At such times, the role of light harvesting is largely assumed by carotenoids and not by bacteriochlorophylls (187). Phototrophs identified in such environments include Chlorobium

limicola, Chromatium, Prosthecochloris, Thiopedia, Thiocystis, and Chlorobium phaeobacteroides.

Primary synthesis of organic material in bodies of water which are not directly exposed to serious pollution, is mediated by the phototrophic bacteria (35). CO_2 is fixed by purple and green sulfur bacteria using H_2S or H_2 as reductant, while acetate and simple organic compounds are assimilated by purple nonsulfur bacteria (139) and some species of the purple sulfur bacteria. These bacteria have been found to contribute significantly to primary production in such habitats (35,42), their contribution ranging from a fraction of a percent to over 80 percent of the total primary production on an annual basis (9).

In addition to being grazed by protozoans in the water column (42), organic constituents of dead cells of phototrophic bacteria are decomposed by fermentative bacteria with the formation of CO_2, H_2, NH_3, organic acids and alcohols. When sufficient sulfate is present, sulfate-reducing bacteria oxidize H_2 with formation of H_2S and acetate which are subsequently used by phototrophic bacteria. Other anaerobic oxidations are performed by methanogenic bacteria which oxidize H_2 and convert CO_2 and acetate to methane. Some of this methane is oxidized in the aerobic region of the water body by aerobic methane oxidizers; the remainder escapes to the atmosphere, constituting a net loss of carbon from the system.

The depth at which the purple sulfur bacteria occur in the water column of stratified lakes varies throughout the day (163). On sunny days they consume H_2S in the upper hypolimnion during the morning and migrate downwards later in the day (128). When this occurs, light becomes the limiting factor (163). Similarly, it has been found that the growth of green sulfur bacteria in stratified lakes is strongly light-limited (8). In holomictic lakes where seasonal stratification can occur, phototrophic sulfur bacteria are located in a narrow horizontal plate where light, H_2S, and minimal O_2 concentration are most favorable for their multiplication (50). Due to the higher tolerance of green sulfur bacteria to H_2S, they are usually located in habitats below the purple sulfur bacteria.

2.3.3.6 Natural Occurrence and Role in Waste Treatment Systems

The natural occurrence of phototrophic sulfur bacteria in systems treating a variety of organic wastes is recorded in the literature (Table 2-9). Their presence is directly attributable to the wide range of substrates metabolized by theml40). These include simple sugars, alcohols, volatile fatty acids (VFA), tricarboxylic acid (TCA) cycle intermediates, and benzoates (139). Odors are generally minimized in systems in which they occur due to their metabolism of H_2S and other odorous compounds. Phototrophic bacteria, by their ability to utilize potentially toxic products of

Table 2-9. Species of phototrophic bacteria identified in waste treatment systems.

Type of Waste	Treatment System	Phototrophic Bacteria	Reference
Rendering plant	Lagoon	Thiopedia rosea Chromatium	34, 35
Petroleum refinery	Lagoon	Chromatium	34, 35
Hide-washing plant	Lagoon	Thiopedia rosea	35
Poultry manure	Lagoon	Thiocystis Thiopedia rosea	35
Municipal	Activated sludge	Chromatium vinosum Thiocapsa roseopersicina	133
Swine manure	Lagoon	Thiopedia rosea Rhodothece Chromatium Thiocapsa roseopersicina	98, 141
Cattle feedlot	Lagoon	Thiopedia rosea	174
Poultry processing	Lagoon	Chromatium	102
Fellmongery	Lagoon	Thiocapsa roseopersicina	33, 98
Meat-packing	Lagoon	Chromatium Thiopedia rosea Thiocapsa roseopersicina	98
Domestic and industrial	Lagoon	Thiopedia rosea Chromatium Thiocapsa roseopersicina	64, 98

anaerobic metabolism, play a beneficial role in the anaerobic degradative process. Studies of lagoons treating organic industrial wastes (38,40,75) have confirmed that purple sulfur bacteria oxidize inorganic sulfur compounds and certain short-chain fatty acids.

As shown in Table 2-9, several species of phototrophic bacteria have been identified in waste treatment systems. Thiocapsa roseopersicina and Chromatium vinosum were found in a domestic waste treatment lagoon (75) which had been overloaded by introduction of highly concentrated potato processing waste. In a study of this lagoon, it was found that levels of acetate, VFA, and sulfides were reduced as the population of phototrophic anaerobes increased. Occurrence of phototrophic sulfur bacteria in a lagoon treating fellmongery wastes (38) correlated with the recorded reduction of 89-98 percent in sulfide levels.

Growth studies on species of Chromatium removed from a poultry processing waste lagoon have indicated an optimum temperature of 26.5° C and optimum pH of 7.5 (116), but growth of these phototrophs has been recorded at temperatures ranging from 10-30° C (38) and pH levels of up to 9.2. In pilot scale waste treatment studies (112) with photobiological systems, COD and sulfide removals of 86 and 85 percent, respectively, were achieved.

2.4. Process Inhibition

Varying levels of certain inorganic and organic substances may have an adverse effect on the anaerobic process. The degree of inhibition by inorganic substances varies depending on whether the substances act singly or in combination with other potential toxicants. Certain combinations of alkaline earth salts have been found to have synergistic effects, while others display antagonistic effects (93). Thus the toxic effects of sodium at 7,000 mg/L may be reduced by 80 percent by addition of 300 mg/L of potassium. It may be completely eliminated by an addition of 150 mg/L of calcium (93).

Digestion of ammonia-rich wastes, such as those from swine and poultry, may be inhibited by either ammonia gas or ammonium ion (37,152). McCarty (107) reported that ammonia gas can become inhibitory at 1500 mg/L to 3000 mg/L total ammonia concentrations and pH greater than 7.4. Ammonia toxicity may be experienced at total ammonia concentrations above 3000 mg/L irrespective of the pH level. Stable methane production was achieved however at total ammonia concentrations in excess of 3000 mg/L (37,52). An investigation of ammonia inhibition in which a urea and acetic acid substrate were used, indicated progressive inhibition to commence at total ammonia concentration of 2000 mg/L, but toxicity did not occur even at total ammonia nitrogen concentration of 7000 mg/L. Nitrates have also

been observed to have a negative effect on methanogenesis (208).

Sulfide has varying effect on methanogenesis. Low concentrations of sulfide may be stimulatory to the process (208), whereas high concentrations can be inhibitory (198). Sulfide concentrations above 200 mg/L are considered toxic to the anaerobic process (107). This is of significance only when sulfides are in soluble form and not when associated with heavy metals. It has also been observed that high concentrations of sulfates inhibited methanogenesis (197). A number of investigators (20,100,198) have proposed that this inhibition results from interspecies competition between the methanogens and sulfate-reducing bacteria for available hydrogen, in which the latter organisms are able to outcompete the former for this substrate.

Levels of organic acids in an anaerobic environment are critical for the efficient operation of the process. McCarty and McKinney (109) found that volatile acid levels below 2000 mg/L were desirable. This observation was confirmed by other investigators (34,60,210). Very small concentrations of heavy metals may be toxic to anaerobic microorganisms in the absence of sulfides with which they form innocuous precipitates (107). Some metals may, however, have a stimulatory effect (164). The impact of a variety of other substances on the anaerobic process has been reported. These include organic priority pollutants

(80), bisulfate (46), trimethylamine (12), sulfur (92), and certain industrial toxicants (130).

Antibiotics and growth promoters are widely used in livestock production. Some of them may adversely affect the anaerobic process; it will be minimized upon acclimation of the microorganisms to the particular chemicals. The detrimental effect of antibiotic lincomycin on the anaerobic process has been recorded (130).

The inhibitory effect of these toxicants will be reflected in reaction rates, and, hence, the kinetic parameters of the anaerobic process (92). While complete allowance cannot be made for all inhibitory substances, several kinetic models now include factors of process inhibition.

2.5 Kinetics of the Anaerobic Process

2.5.1 Basic Considerations

In a completely mixed anaerobic reactor, various steps of the staged sequential biodegradative process are assumed to occur simultaneously. Consequently, overall kinetics of this complex process are considered to be controlled by the kinetics of a rate limiting step. Identification of this rate-limiting step and determination of its kinetic characteristics are considered essential to the development of overall process kinetics.

The theory of growth kinetics in biological systems, is based on growth rate, growth yield, and the relationship between growth rate and an essential nutrient. Mathematical descriptions of these relationships may be derived, thus enabling development of kinetic equations or mathematical models, for describing microbial activities under particular growth conditions.

The Monod model (121) has successfully described the kinetics of biological waste treatment systems and has provided the basis for several kinetic models of the anaerobic digestion process. This model describes the growth of homogeneous cultures on simple substrates but not heterogeneous cultures on complex substrates as found in anaerobic environments. In spite of apparent limitations, the Monod model has been adapted to the anaerobic digestion kinetics of sewage sludge and animal manures (65,96). Prediction of maximum biological activity and washout of microbial cells from a reactor are possible with this model. One other advantage of this type of model is that the kinetic parameters (microorganism maximum specific growth rate and half-velocity constant) fully describe the microbial processes, although different parameters must be used for short and long retention times (31,32). A disadvantage of Monod type models is that the kinetic parameters cannot be obtained for certain complex substrates (136).

Disadvantages of Monod models were overcome with the development of various forms of first-order kinetic models (1,2,3), which were simple to use and gave good fit of experimental data. They are, however, limited in their ability to predict the conditions for maximum biological activity and system failure.

2.5.2. Relationship Between Microbial Growth and Substrate Utilization in Batch Culture

In the logarithmic phase of bacterial growth, the growth rate of organisms can be expressed by the equation

$$dX/dt = \mu X \qquad (2\text{-}9)$$

where X = organism concentration, mass/unit volume

t = time

μ = specific growth rate, time^{-1}

Accounting for the effect of endogenous respiration on the overall growth rate, equation 2-9 becomes

$$dX/dt = (\mu - b)X \qquad (2\text{-}10)$$

where b = endogenous decay coefficient, time^{-1}

dX/dt = net growth rate of microorganisms per unit volume of reactor, mass/volume-time

Substrate utilization rate is considered proportional to the biomass present, as expressed by the equation

$$dS/dt = qX \tag{2-11}$$

from which

$$q = \frac{dS/dt}{X} \tag{2-12}$$

where dS/dt = substrate utilization rate, mass/volume-time

q = specific substrate utilization rate, time^{-1}

True growth yield, Y_T, is defined as

$$Y_T = \frac{\text{weight of organisms formed}}{\text{weight of limiting substrate utilized}}$$

Mathematically,

$$Y_T = dX/dS \tag{2-13}$$

where Y_T = growth yield constant, mass/mass

Combination of equations 2-10, 2-11 and 2-12 provides the relationship between biological growth and substrate utilization

$$dX/dt = Y_T(dS/dt) - bX \tag{2-14}$$

Hence

$$q = \mu/Y_T \tag{2-15}$$

Rate of substrate utilization may also be related to concentration of microorganisms in the reactor and concentration of the growth-limiting substrate surrounding the organisms as

$$\frac{dS}{dt} = \frac{kSX}{K_s + S} \qquad (2\text{-}16)$$

where k = maximum rate of substrate utilization per unit of microorganisms.

S = concentration of growth-limiting substrate surrounding the microorganisms, mass/volume

K_s = half-velocity constant, equal to the substrate concentration when $(dS/dt)/X = (1/2)k$, mass/volume

This expression is in similar form to the Monod equation (121) which describes the rate of bacterial growth as a function of substrate concentration surrounding the microorganisms

$$\mu = \mu_{max} \frac{S}{K_s + S} \qquad (2\text{-}17)$$

where μ = specific growth rate, time^{-1}

μmax = maximum specific growth rate, time^{-1}, constant

K_s = half velocity constant

Combining equations 2-14 and 2-16 yields the expression

$$\mu_n = \frac{Y_T kS}{K_s + S} - b \qquad (2\text{-}18)$$

where μ_n = $(dX/dt)/X$, net specific growth rate

When S is very much greater than K_s in equation 2-16, K_s can be neglected and the equation becomes

$$dS/dt = kX \qquad (2\text{-}19)$$

When S is very much less than K_s, S in the denominator can be neglected and the equation becomes

$$dS/dt = KSX \qquad (2\text{-}20)$$

where $K = k/K_s$, specific substrate utilization rate constant, volume/mass.time

2.5.3. Completely-Mixed Continuous Culture Model Without Recycle

2.5.3.1. Microbial Growth

Consider the schematic of a continuous culture reactor shown in Figure 2-10. A materials balance for bacteria yields

microorganism accumulation within reactor	=	rate of microorganism inflow	+	net growth of microorganism within reactor	-	net rate of microorganism outflow

or stated directly

accumulation = inflow + net growth - outflow

$$\text{i.e. } (dX/dt)V = QX_o + \mu_n VX - QX \qquad (2\text{-}21)$$

where V = volume of microbial culture in reactor

X_o = microorganism concentration in influent, mass/volume

X = microorganism concentration in reactor mass/volume

Q = flowrate, volume/time

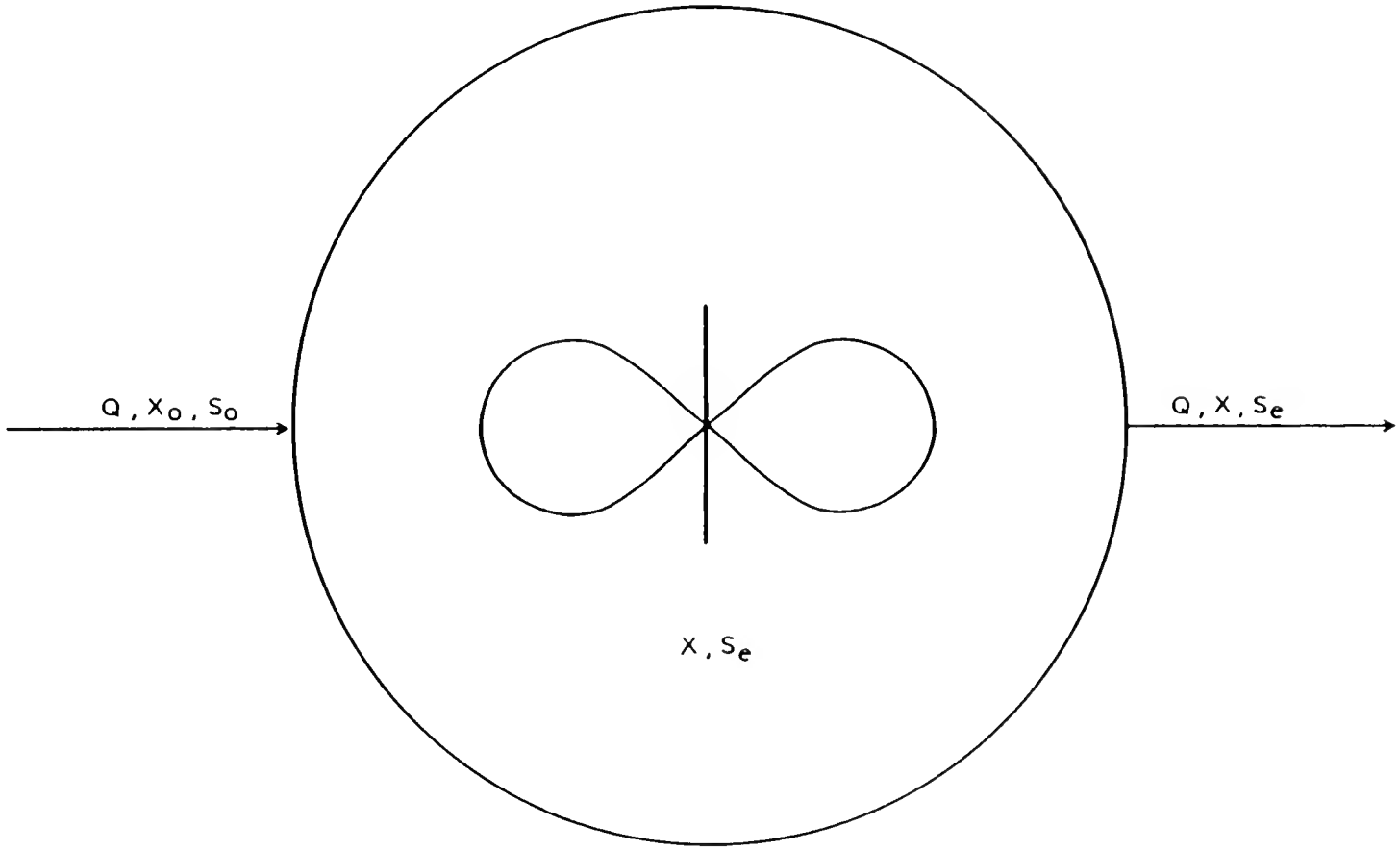

Figure 2-10. Completely-mixed no solids recycle.

Neglecting microorganisms in influent, assuming steady-state conditions (dX/dt = 0), and substituting for μ_n from equation 2-18, equation 2-21 reduces to

$$\frac{Q}{V} = \frac{Y_T k S_e}{K_s + S_e} - b = \frac{1}{\theta} \qquad (2\text{-}22)$$

where θ = hydraulic retention time, V/Q

At steady state, the specific growth rate is equal to the dilution rate, and

$$\mu_n = \frac{1}{\theta} = \frac{1}{\theta_c} = \frac{Y_T k S_e}{K_s + S_e} - b \qquad (2\text{-}23)$$

where θ_c = biological solids retention time, time

$1/\theta$ = dilution rate, time^{-1}

from which we obtain the expression

$$\frac{1}{\theta_c} = Y_T \frac{(dS/dt)}{X} - b \qquad (2\text{-}24)$$

$$= Y_T q - b \qquad (2\text{-}25)$$

From equation 2-24

$$\frac{dS}{dt} = \frac{1}{Y_T} \cdot \frac{X}{\theta_c} + bX \qquad (2\text{-}26)$$

Net microbial growth may be described by the expression (96)

$$\frac{dX}{dt} = Y_{obs} \cdot \frac{dS}{dt} \qquad (2\text{-}27)$$

where Y_{obs} = variable observed yield coefficient.

From equation 2-27

$$Y_{obs} = \frac{(dX/dt)}{(dS/dt)} \qquad (2\text{-}28)$$

multiplying the right hand side of expression by X/X gives

$$Yobs = \frac{\mu}{q} \qquad (2\text{-}29)$$

Substituting dS/dt from equation 2-27 in equation 2-26

$$\frac{1}{Y_{obs}} \cdot \frac{dX}{dt} = \frac{1}{Y_T} \cdot \frac{X}{\theta_c} + bX \qquad (2\text{-}30)$$

This expression reduces to

$$\frac{1}{Y_{obs}} = \frac{b\theta_c}{Y_T} + \frac{1}{Y_T} \qquad (2\text{-}31)$$

The substrate utilized by an organism may be considered to comprise a variable portion for biosynthesis and a relatively constant portion for maintenance. The total specific substrate utilization rate can therefore be expressed as

$$q = a_{1\mu} + a_2 \qquad (2\text{-}32)$$

where a_1 = substrate utilization to form a unit of biomass

μ = specific growth rate of biomass

a_2 = substrate utilized for maintenance function per unit biomass per unit time, time^{-1}

q = specific substrate utilization rate, time^{-1}

Substituting for q in equation 2-32 from equation 2-29 gives

$$Y_{obs} = \frac{1}{a_1 + a_2/\mu} \qquad (2\text{-}33)$$

2.5.3.2. Substrate Utilization

A similar materials balance may be written for substrate within the reactor

change within the reactor = influent - consumption - effluent

or $$V(dS/dt) = QS_o - V(KXS_e) - QS_e \qquad (2\text{-}34)$$

where KXS_e = substrate consumed by organisms

At steady state dS/dt = 0, and equation 2-34 becomes

$$Q(S_o - S_e) = V.KS_e$$

and $$\frac{Q(S_o - S_e)}{XV} = KS_e = q \qquad (2\text{-}35)$$

2.5.4 Anaerobic Kinetic Models

The anaerobic digestion process has been described by several kinetic models, which have been developed to optimize gas production (30) rather than waste treatment, as is the case with activated sludge kinetics. None of these models have been developed for the treatment of animal manures by the anaerobic photosynthetic process. McFarlane and Melcer (111) have, however, demonstrated the

applicability of Monod-type kinetics to the treatment of industrial wastes by the anaerobic photosynthetic process.

Andrews (1,2) was among the first to introduce the dynamic modeling of the anaerobic digestion process. He incorporated an inhibition function into the model by considering un-ionized volatile acids as the rate-limiting substrate and inhibitory agent. This approach yielded the expression

$$\frac{\mu}{\mu_{max}} = \frac{1}{1 + \frac{K_s}{UVA} + \frac{UVA}{K_i}} \qquad (2\text{-}36)$$

where μ = specific growth rate, time^{-1}.

μ_{max} = maximum specific growth rate, time^{-1}.

K_s = saturation constant, mass/volume.

K_i = inhibition coefficient of un-ionized volatile acids, mass/volume.

UVA = concentration of un-ionized volatile acids, mass/volume.

This model was further developed and expanded by Andrews and Graef (3), who considered the effect of interactions between volatile acids, pH, alkalinity, gas production rate and gas composition on the process. This resulted in the development of a model capable of predicting process performance under transient conditions. Municipal and industrial wastes were used as the influent material in the development of this model.

Using computer simulation, Hill and Barth (65) modified the model of Andrews and Graef (3) by substituting animal manure, with its higher content of organic material and nitrogen, as the influent substrate and considering the inhibitory effect of free (un-ionized) ammonia on the methane-producing bacteria. The resulting expression was

$$\frac{\mu}{\mu_{max}} = \frac{1}{1 + \frac{K_s}{UVA} + \frac{UVA}{K_i} + \frac{NH_3}{K_{i2}}} \qquad (2\text{-}37)$$

where NH_3 = concentration of un-ionized ammonia, mass/volume.

K_{i2} = inhibition coefficient for ammonia, mass/-volume.

The Monod (121) kinetic model has been adapted to describe anaerobic digestion kinetics of sewage sludge (96). The disadvantages of this model, as noted above were minimized with the development of first-order kinetic models (58,59). Applying Monod (121) kinetics, Ghosh and Pohland (55) investigated the kinetics of substrate assimilation and product formation in anaerobic digestion. The Contois (36) kinetic model was adapted by Chen and Hashimoto (30) to describe the kinetics of methane fermentation in the form

$$B_v = \frac{B_o S_o}{\theta}\left[1 - \frac{K}{\theta\mu_m - 1 + K}\right] \qquad (2\text{-}38)$$

where B_v = volumetric methane production rate L CH_4/L fermenter.day.

S_o = influent total volatile solids (VS) concentration g/L.

B_o = ultimate CH_4 yield, L CH_4/gm VS added

θ = retention time, day.

μ_m = maximum specific growth rate of organisms, day $^{-1}$

K = kinetic parameter, dimensionless.

This model was used to predict B_v of pilot and full-scale systems fermenting livestock wastes at 35, 55 and 60° C. The equation relates the daily volume of methane produced with loading rate (S_o /θ), material biodegradability (B_o) and kinetic parameters μ_m and K. For livestock wastes, B_o depends on type, animal ration and age of the manure, the collection and storage method, and the amount of foreign material incorporated in the manure.

In a general presentation, Lawrence (94) outlined a kinetic approach to the design of biological waste treatment processes employing suspended cultures of microorganisms in completely mixed process configurations. The relationships developed for concentrations of effluent waste (Equation 2-38) and microbial biomass in the reactor (Equation 2-39) would be applicable to anaerobic digestion. These expressions are

$$S_1 = \frac{K_s [\ 1 + b(\theta_c)\ ]}{\theta_c (Yk - b) - 1} \qquad (2\text{-}39)$$

$$X = \frac{Y (S_o - S_1)}{1 + b\theta_c} \qquad (2\text{-}40)$$

where S_1 = effluent waste concentration, mass/volume.

K_s = half velocity coefficient, mass/volume.

b = microorganism decay coefficient, time^{-1}

θ_c = biological solids retention time, time.

Y = growth yield coefficient, mass/mass.

k = maximum rate of substrate utilization per unit weight of microorganisms, time^{-1}

X = microbial mass concentration, mass/volume.

S_o = influent waste concentration, mass/volume.

In a comparative evaluation of the kinetic constants reported for methanogenic bacteria by several researchers, Scharer and Moo Young (148) found wide variations in the parameters. These discrepancies were attributable to both experimental conditions and methods of data analysis used. They observed that kinetic information determined from single substrates could not be used for predicting methane generation from complex substrates.

The development of mathematical models and simulation techniques for anaerobic digestion of animal waste have been described by Hill and Barth (65), and Hill and Nordstedt (67). These models were later refined (64,68) to reflect new assumptions, thus enabling more accurate prediction of process to be made.

CHAPTER 3
MATERIALS AND METHODS

3.1 Rationale for Experimental Design

The present research was designed to generate data for the determination of kinetic parameters which influence phototrophic biomass production and substrate uptake in a swine waste medium. These parameters are represented in Equations 2-33 and 2-35.

Two laboratory-scale anaerobic reactors, one illuminated, and the other nonilluminated, were operated in parallel in order to assess the impact of bacterial photosynthesis on the anaerobic digestion process. In such a configuration the nonilluminated reactor would yield results consistent with a conventional anaerobic digester, and would be considered as the control. Retention times, which ranged from 5 to 30 days were selected on the basis of published growth characteristics of methanogenic bacteria and on the retention times normally used for standard rate digesters. Uniform distribution of the medium throughout the reactor was achieved by continuous mixing with magnetic stirrers, ensuring that the bacteria within the reactor would always be in intimate contact with the medium, and that the biological solids retention time (SRT) was equal to

the hydraulic retention time. A low mixing speed was selected to achieve gentle continuous turnover of the contents of the reactor without introduction of high shear forces.

The medium was blended from raw swine waste collected directly from floors of the pig barns. This method of preparation effected greater control over the solids concentration used in the experiments, avoiding dilution which would be experienced by flushing of the wastes. It also eliminated any prior fermentation which would occur in storage tanks. Before being blended to the required concentration, the waste was screened to remove coarse cellulosic or inorganic materials, yielding a more easily biodegradable medium. The experiments were started with waste of total solids concentration in the range 0.4 to 0.6 percent, this range having been found (45) to be suitable for batch growth of phototrophic bacteria. Waste with volatile solids (VS) concentration of 1.0 percent was used for continuous loading of the reactors. Selection of this concentration was based on the results of earlier batch studies (45).

The contents of both reactors were maintained at a temperature of 27 1 C in all experiments. Phototrophic sulfur bacteria were observed to grow very well at this temperature (45). The pH was not controlled in any of the experiments conducted, but was allowed to vary as dictated

by the buffering capacity of the system. The reactors were kept airtight to exclude oxygen, and sulfide levels were maintained above 20 mg/L in the reactors by addition of sodium sulfide as found necessary. The addition of sulfide ensured that this nutrient did not become limiting during the experiments. Illumination was provided by 120 watts of incandescent lighting. Optimum light level was not known at the time the experiments were designed.

Several parameters were measured and used in interpreting the performance of the reactors. Measurements were made throughout the operation of the reactors, but particular emphasis was placed on sampling and analysis once a relatively stable level of bchl _a_ had been attained. This was considered to be an indication of steady state conditions in the reactors. Measurement of pH was made to assess the buffering capability of illuminated and nonilluminated systems. Bacterial growth and biomass production were monitored by measurement of bacteriochlorophyll _a_ (bchl _a_) concentration. Biomass productivity was also determined by measurement of total, volatile and suspended solids, and protein. Chemical oxygen demand and biochemical oxygen demand were used in monitoring substrate uptake in the system. Nitrogen (N) and phosphorus (P) were determined in order to compare nutrient uptake in both reactors. Gas quantity and quality were monitored to determine impact of phototrophic bacteria on biogasification.

3.2 Summary of Investigations

The performance of two bench-scale anaerobic reactors, an illuminated experimental reactor and a nonilluminated control reactor was monitored at SRTs of 5, 7, 8.5, 10, 15, 20, and 30 days. The reactors were operated at a temperature of 27 ± 1°C and were loaded with screened and blended swine waste having a volatile solids concentration of 1.0 ± 0.1 percent. Parameters measured included temperature, pH, bacteriochlorophyll *a*, chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total solids (TS), volatile solids (VS), ammonia-nitrogen (NH_3-N), total kjeldahl-nitrogen (TKN), total suspended solids (TSS), total sulfide, total phosphorus (P), and gas quantity and quality.

3.3 Experimental Apparatus

The apparatus is shown schematically in Figure 3-1. Two identical 4.0 L glass bottles, each sealed with a rubber stopper, were used as anaerobic reactors. The stoppers were fitted with inlet/outlet, venting, and gas transfer tubing. Each was also fitted with a thermometer. The reactors were operated with liquid volume of 3.5 L. The control reactor was completely covered with aluminum foil to exclude light wherezas the experimental reactor was continuously exposed to illumination.

Illumination was supplied by two banks of incandescent lights each containing 2 x 30-W floodlights (Westinghouse

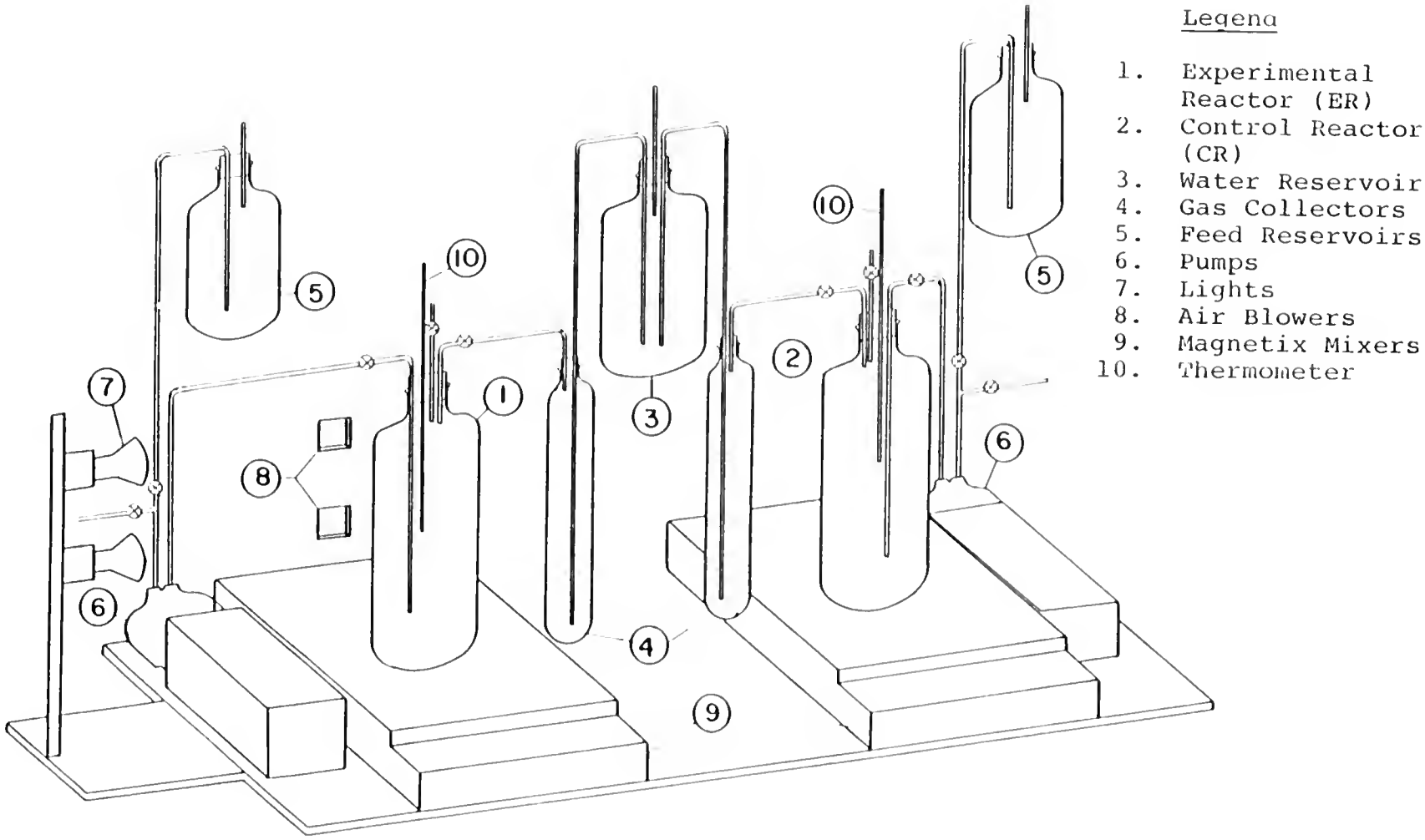

Figure 3-1. Schematic diagram of experimental apparatus.

30R20, Westinghouse Electric Corp., Bloomfield, New Jersey). The lights in each bank were 12 cm apart vertically, with the lower light being 12 cm above the base of the reactor. The banks were spaced 135 degrees apart horizontally from each other, and 30 cm from the side of the reactor.

The illuminated reactor was cooled by Dayton Model 4C004A air blowers (Dayton Electric Manufacturing Co., Chicago, Illinois) placed 12 cm from the reactor, one 8 cm and the other 20 cm above its base. Each blower had a maximum capacity of 36 L/s at a speed of 2880 rev/min. Airflow control was effected by a sliding window arrangement which allowed the temperature to be maintained within the required range of 27 $\pm$ 1°C. The required temperature of the non-illuminated reactor was achieved by heating with a single 150-W incandescent floodlight supported on an adjustable base, which enabled its distance from the reactor to be varied as necessary.

Continuous mixing was accomplished by magnetic stirrers (Fisher Thermix Stirrer Model 620T, Fisher Scientific Company, Fair Lawn, New Jersey). Gas was collected by displacement of an acidified 5 percent solution of sodium chloride from 1200 ml nominal capacity graduated gas collector bottles equipped with septa in the stoppers for gas sampling.

Reactors were loaded and unloaded by Masterflex peristaltic pumps (Cole-Parmer, Chicago, Illinois), as described in section 3.4.2.

3.4 Materials

3.4.1 Substrate

The substrate was swine waste obtained from the University of Florida's Swine Research Unit (SRU). The waste was collected from solid floors of barns housing finishing hogs. These pigs were kept on a high grain finishing ration of corn and soybean meal. The composition of the feed at any particular time depended on the experiments being conducted by the SRU. Details of the ration are included in Table 3-1. Following collection, the waste was dispensed into 250 mL containers and frozen until ready for use. When required, the waste was thawed at 4/C, mixed with tap water and screened by passing through a 105 μm screen (U.S.Standard Sieve No. 140, Soiltest Inc., Evanston, Illinois), analyzed, and diluted with tap water to a concentration of 1.0 $\pm$ 0.1 percent VS. Typical characteristics of the waste used in these experiments are shown in Table 3.2.

Table 3-1. Composition of grower/finisher ration used at the University of Florida's Swine Research Unit during investigations.

Ingredients	Percent
Basic	
Corn Meal	77 - 88
Soybean Meal	9.4 - 20
Dynafos	0.1 - 2.8
Limestone	0.8 - 1.0
Iodized Salt	0.2 - 0.5
Trace Mineral	0.1
Vitamin Mix	0.1
Additives	
Antibiotics	0.15
Selenium	0.05 - 3.0
Potassium	0.5 - 1.0
Magnesium	1.4 - 2.8
Lysine	0.1 - 0.3

Note: Additives were not all used at the same time.

Table 3-2. Principal characteristics of swine waste collected from the confinement units.

Parameter	Unit	Concentration
COD	g/L	17.8 - 21.6
BOD_5	g/L	6.1 - 8.3
Kjeldahl-N	g/L	0.86 - 1.015
Ammonia-N	g/L	0.20 - 0.45
Total Phosphorus	g/L	0.413 - 0.566
Total Solids	g/L	12.1 - 13.53
Total Volatile Solids	g/L	9.1 - 10.8
Total Suspended Solids	g/L	8.5 - 10.6
Total Sulfide	g/L	0.04 - 0.07
pH		6.8 - 7.0
Temperature	°C	24 - 26

3.4.2 Bacterial Inocula

3.4.2.1 Phototrophs

Phototrophic bacterial inoculum for each experiment consisted of a blend of equal parts of laboratory-cultured organisms and effluent obtained from the anaerobic lagoon at the University of Florida's Swine Research Unit. This lagoon, which receives waste from an average population of 260 pigs housed on solid concrete slab and slatted floors, normally contained a dense population of phototrophic sulfur bacteria. Their presence was recognized by the vivid red to purple-pink color imparted to the lagoon.

3.4.2.2 Methanogens and Other Anaerobes

Methanogens and other anaerobes used as inoculum were obtained from a 20 m^3 capacity standard rate anaerobic digester treating swine waste. This digester was located at the SRU.

3.5 Experimental Methods

3.5.1 Start-up Batch Cultures

The experiments were conducted in two series. The first series extended from September 1983 to March 1985, and included SRTs of 5, 7, 10, 15, 20, and 30 days. Each trial consisted of a batch phase which continued until onset of the stationary period of phototrophic bacterial growth,

which was indicated by a levelling-off of the growth curve. The batch phase was followed by a continuous-loading phase during which the reactors were loaded daily at a volumetric rate consistent with the retention time of the trial. Both experimental (illuminated) and control (nonilluminated) reactors were operated throughout each trial in this series.

The second series of trials extended from April 1985 to September 1985, and included SRTs of 8.5, 10, 15, 20, and 30 days. The 10-d SRT and 20-d SRT trials involved an initial batch phase followed by a continuous loading phase, as in the first series. However, only experimental reactors were operated; there were no controls. Following attainment of steady state conditions, the loading rate of the 10-d SRT reactor was increased to give a SRT of 8.5-d, and the loading rate of the 20-d SRT was increased to give a SRT of 15-d. A final trial at a SRT of 30-d was conducted by reducing the loading rate of the 15-d SRT reactor after it had reached steady state.

The growth medium of experiments which started with a batch growth phase comprised 62.5 percent v/v of swine waste (prediluted to 0.4-0.6 percent total solids concentration), 25 percent v/v of phototrophic bacterial inoculum, and 12.5 percent v/v of methanogenic inoculum. Temperature and pH were recorded, and 3.5 L of this growth medium was dispensed to each reactor. Initial total solids and volatile solids concentrations were also measured.

Gas production and temperature were monitored daily, and bacteriochlorophyll a (bchl a) concentration and pH were measured at intervals of two days, or as considered appropriate. Upon attainment of the stationary growth phase of phototrophic bacteria, the experiment was continued by loading the reactors once daily with swine waste.

3.5.2 Continuous Mode

Solids residence times (SRTs) of 5, 7, 8.5, 10, 15, 20, and 30 days with respective volumetric loading rates of 700, 500, 411.8, 350, 233.3, 175, and 116.7 mL/d were used in these experiments. Each reactor was loaded and unloaded on a daily basis with feedstock which was kept refrigerated and brought to room temperature prior to loading. Each reactor was first unloaded by pumping the required volume of effluent from the reactor. When enough gas was available in the gas collectors, it was drawn into the reactor simultaneously as the effluent was withdrawn, to reduce the possibility of air being drawn into the system. The measured volume of feedstock was then pumped into the reactor from the reservoir, simultaneously replacing the gas which had been drawn in during the unloading process. This arrangement ensured that the space above the liquid in the reactors was saturated with reactor gas. This was not always possible, and it sometimes became necessary to draw a certain volume of air into the reactor during unloading due

to shortage of reactor gas. This volume was subsequently displaced from the reactor during loading.

The volume of gas produced by each reactor in the 24-hour interval between loading was monitored daily by recording the volume of water displaced from a graduated cylinder. Reactor temperature was monitored at least twice daily; pH and bchl *a* concentration were generally measured at intervals of two days, except in situations where it became necessary to measure these parameters daily, or at other intervals.

Gas was sampled and analyzed at intervals during both batch and continuous phases. The samples were withdrawn via a septum in the stopper of the gas collector using 1 cc Tuberculin 26G 3/8 disposable hypodermic syringes (Becton, Dickinson and Company, Rutherford, New Jersey 07070). The gas-filled syringes were sealed immediately upon withdrawal from the septum by inserting the tip of the needle into a rubber plug. They were removed from this plug immediately prior to injection of the gas sample into a gas chromatograph.

Phototrophic bacterial activity was monitored by bchl *a* concentration. Upon attainment of steady state conditions in the illumiated reactor, which was identified by a leveling-off of bchl *a* concentration over a period of several days, analyses were carried out on samples from each reactor. Four complete sets of analyses were carried out for

each steady state condition over a period of 8 days. With the exception of phosphorus, analyses of all samples were initiated within one hour of withdrawal from the reactors. Samples for phosphorus determination were filtered and acidified to pH 2.0 and stored at 4°C until analysed. Gas samples were also analysed for methane content at these times.

3.6 Analytical Techniques

3.6.1 Bacteriochlorophyll a

Bchl a was measured using a modified version (44) of the procedure described by Siefert et al. (151). For analysis, 10 mL of a ten-fold diluted sample was centrifuged for 15 minutes at 2400xg and the centrate decanted. The pellet was washed by adding 10 mL of distilled water to the centrifuge tube, resuspending the pellet by manual shaking and centrifuging again for 15 minutes. The centrate was decanted and 10 mL of a 7+2 (v/v) solution of acetone/methanol was added to the pellet in the centrifuge tube and the tube shaken vigorously to resuspend the particulate matter. Extraction was carried out in the dark at room temperature for 30 minutes. The sample was then centrifuged again for 15 minutes, and absorbance of the centrate was measured at a wavelength of 770 nm, with an additional reading at 850 nm to correct for turbidity. The bchl a concentration, in mg/L, was computed from the following equation:

$$\text{bchl } \underline{a} = 12.1 \times (D_{770} - D_{850}) \times F \qquad (3\text{-}1)$$

where D_{770} = absorbance at 770 nm, D_{850} = absorbance at 850 nm, and F = (v/V) x l in which v = volume of acetone/methanol extract (mL), V = sample volume (mL), and l = path length (cm).

3.6.2 Sulfide

Sulfide concentrations were measured by the methylene blue method (Section 427C) of Standard Methods (166). Samples were analyzed immediately following collection. A 0.5 mL volume of an amine-sulfuric acid reagent was added to 7.5 mL of sample followed by addition of 0.15 mL $FeCl_3$ solution. The presence of sulfide was indicated by the appearance of a blue color. Five to ten minutes were allowed for color development then 1.6 mL of diammonium hydrogen phosphate solution was added. This produced a white precipitate leaving a clear blue supernatant for color determination, the absorbance of which was read at 664 nm. A blank was prepared at each analysis and used for standardization. This was done by substituting 0.5 mL 1 + 1 H_2SO_4 for the amine-sulfuric acid reagent in a measured 7.5 mL sample and following all other procedures as above. Sulfide concentrations were determined from a standard curve. At least one standard was analyzed with each batch of samples.

3.6.3 Protein

Crude protein was computed on the basis that protein comprised 16 percent nitrogen. It was determined from the relationship:

$$\text{Crude protein} = 6.25 \times \text{particulate TKN.} \qquad (3\text{-}2)$$

3.6.4 BOD_5, COD, TS, VS and TSS

These parameters were determined by the procedures given in Standard Methods (166). BOD_5 was determined by the procedure in Section 507, using the azide modification of the titrimetric iodometric method (Section 421B), and the dichromate reflux method (Section 508A) was used for COD determinations. TS, VS and TSS were determined by the procedures of Sections 209A, 209E and 209D, respectively. A double filter technique entailing use of a 1.2 μm pore size Whatman GF/C filter (Fisher Scientific Company, 711 Forbes Avenue, Pittsburgh, Philadelphia 15219) overlying a 0.45 μm pore size GN-6 Metricel membrane filter (Gelman Sciences, Inc., Ann Arbor, Michigan 48106), was used in preparation of the filtered samples. A similar technique was used for particulate determinations.

3.6.5 Kjeldahl-N, NH_3-N and Total P

Total Kjeldahl-nitrogen and ammonia-nitrogen were determined by adaptation (44) of the micro-Kjeldahl procedure (Sections 351.2 and 350.2 respectively) of the EPA's Methods for Chemical Analysis of Water and Wastes (183). Total phosphorus was determined by the procedures outlined in Standard Methods (166), using persulfate digestion (Section 424C-III) and ascorbic acid (Section 424F) methods.

3.6.6 pH

pH was monitored by an electrode-analyzer system (701A, Orion Research Inc., Cambridge, Massachusetts).

3.6.7 Absorbance

Optical density at wavelengths specified by the colorimetric procedures was measured by absorption spectrometry (Bausch and Lomb Spectronic 70, Bausch and Lomb, Marietta, Georgia).

3.6.8 Gas Quantity and Quality

Daily gas production was measured by water displacement. Gas samples were analysed on a Gow Mac Series 550 gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) (Gow Mac Instrument Company, Madison, New Jersey). Bottled methane and carbon dioxide gas were used

for preparation of standard curves. Peak heights were recorded on a nonintegrating chart recorder (Omniscribe Model 5710-5, Houston Instrument Company, Bellaire, Texas). Operating conditions of the GC are listed in Table 3-3.

Table 3-3. Operating conditions for chromatographic analysis of gas samples.

Parameter	Characteristic
Column	6.35 mm x 2.44mm
Packing	Poropak Q
Support	50 - 80 Mesh
Detector	Thermal conductivity (TCD)
Carrier Gas	Helium
Gas flowrate	70 ml/min
Gas pressure	28124 kg/m^2
Injection port temperature	90 C
Column temperature	75 C
Detector temperature	135 C
Bridge Current	195 mA

CHAPTER 4
RESULTS

4.1. Identification of Phototrophic Bacteria

The illuminated laboratory cultures varied in color from a vivid purple-red to reddish-brown at peak bchl *a* concentrations. Microscopic examination of these cultures revealed small non-motile coccoid cells approximately 1-2.5 μm in diameter as the dominant species of phototrophic bacteria. These were tentatively identified as *Thiocapsa roseopersicina*. Under the microscope the dominant species of phototrophs in the lagoon effluent used as inoculum were observed to be in sheet-like cell clusters which is a typical characteristic of *Thiopedia rosea*.

Enrichment of samples from the lagoon and the laboratory reactors were carried out at Southern Illinois University by Professor M.T. Madigan. An enrichment medium containing citric acid was used. Examination of the enriched cultures from the lagoon confirmed the presence of cells in tetrads which were free of gas vacuoles and were encased in a slimy outer layer. These were considered to be *Thiopedia*/*Thiocapsa* type organisms. Examination of an enriched laboratory culture indicated the presence of spherical cells with no evidence of internal sulfur

granules. These were considered to be sulfur-free cells of Thiocapsa. In addition the laboratory sample contained large numbers of organisms similar in appearance and characteristics to the nonsulfur purple bacteria species Rhodopseudomonas palustris and Rhodopseudomonas sphaeroides (Professor M.T. Madigan, personal communication 1985). This enrichment was made from the reactor contents a few days after termination of the final experiment. Indications are that sulfide had been depleted at the time the sample was taken, leading to the proliferation of the nonsulfur species of bacteria.

4.2 Temporal Variation of Phototrophic Bacterial Population, Gas Production and pH During Experimental Trials

4.2.1 Experimental Series

Variation of bchl a, gas production and pH during the experimental trials is described in this section. The trials were conducted in two series, the first of which lasted from September 1983 to March 1985, and the second from April 1985 to September 1985. Most of the trials consisted of an initial batch phase followed by a continuous loading phase (batch/continuous mode trials). In the first series of experiments all trials were of this type and these results are described first. The retention times for some trials were achieved by a change of loading rate between one continuous phase and another (continuous/continuous mode

trials). Most of the experiments in the second series were of this type, and these results are presented separately. It should be noted that in the second series, which also included batch/continuous trials, no control reactors were operated.

4.2.2 Batch/Continuous Mode Trials

4.2.2.1 5-d SRT

Plots of bchl *a*, gas production and pH in the experimental and control reactors during the 5-d SRT trial (Series 1) are shown in Figure 4-1. Following inoculation, the batch phase was continued for 22 days. From an inoculum level of 16.3 mg bchl *a*/L, the bchl *a* concentration attained a maximum level of 58.5 mg/L in the experimental reactor (ER) while declining to 10.9 mg/L in the control reactor (CR). Gas production during the batch phase peaked at 25.8 mL/h in the ER and 28.9 mL/h in the CR. Maximum pH values attained during this phase were 7.2 and 7.0 in the ER and CR, respectively.

Upon commencement of continuous loading, bchl *a* concentrations declined exponentially in both reactors until the trial was terminated. Final bchl *a* levels were 2.42 mg/L and 1.06 mg/L in the ER and CR respectively. pH fell in both reactors also, ending at a value of 5.9. In the continuous mode, gas production in the ER reached a maximum of 16.6 mL/h 3 days after commencement of daily loading but

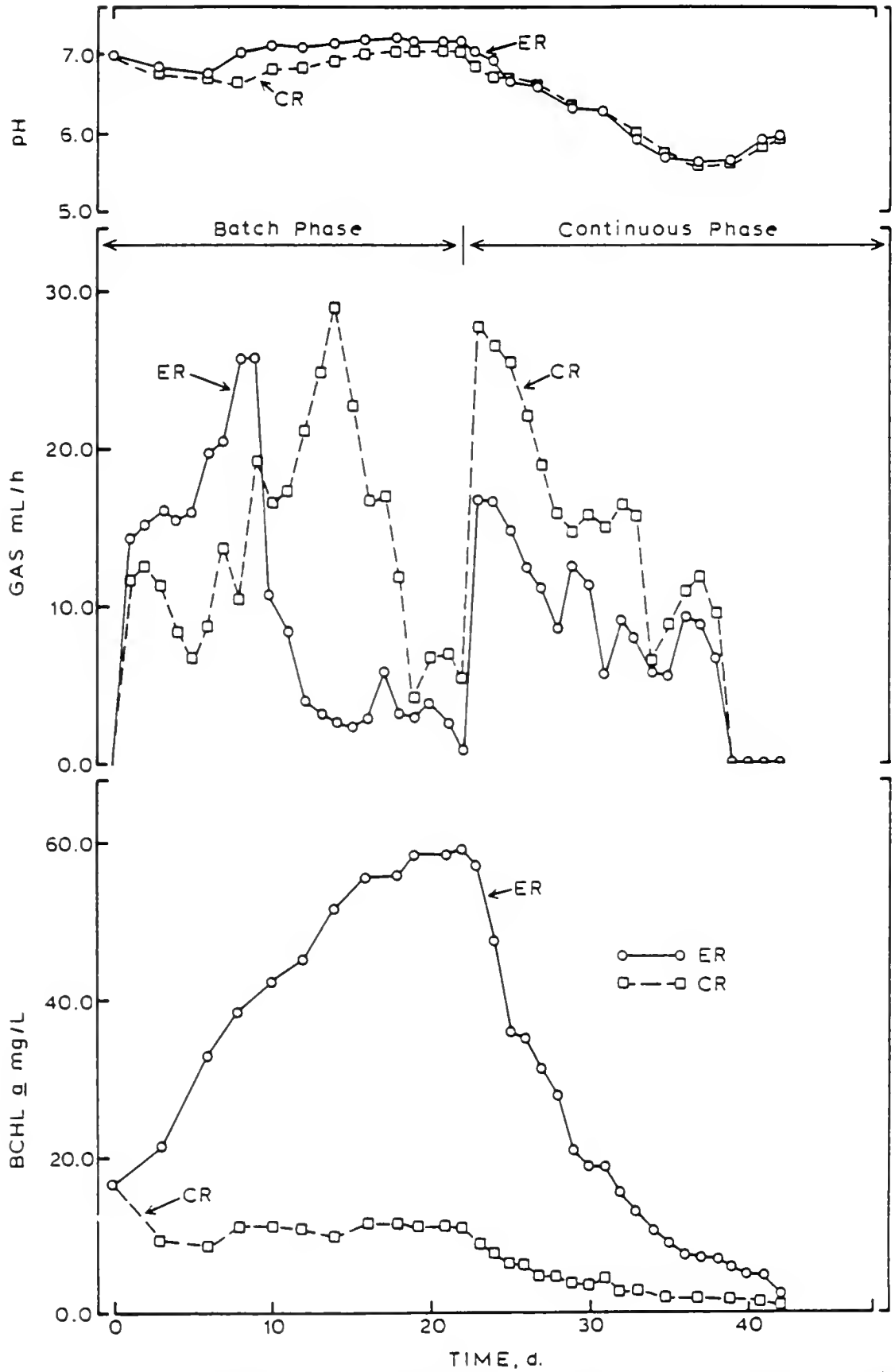

Figure 4-1. Temporal variation of bchl a, biogas production, and pH during the 5-d SRT trial, series 1. ER = experimental (illuminated) reactor, CR = control (nonilluminated) reactor).

subsequently declined to zero. Gas production in the CR increased to a peak of 27.8 mL/h one day after commencement of daily loading but declined to zero 16 days later.

4.2.2.2 7-d SRT

Bchl a, gas production and pH are plotted versus time in Figure 4.2. The initial bchl a concentration was 8.5 mg/L. The bchl a concentrations at the end of the batch phase were 59.3 mg/L in the ER and 6.7 mg/L in the CR. Upon commencement of daily loading, these values declined to 4.2 mg/L and 0.6 mg/L in the ER and CR, respectively, over a period of 19 days.

In the batch phase, gas production peaked at 20.2 mL/h in the ER and 32.8 mL/h in the CR after 12 days. Gas production then declined sharply, but this decline was arrested on commencement of continuous loading. During the latter phase, gas production peaked at 39.5 mL/h in the ER and 64.1 mL/h in the CR. pH ranged between 6.6 and 7.2 in both reactors throughout the experiment.

4.2.2.3. 10-d SRT

Two experimental trials involving both batch and continuous phases were conducted at the 10-d SRT. In the first trial (Figure 4-3), bchl a in the ER increased from an initial level of 9.5 mg/L to a peak of 45.6 mg/L at the end of the batch phase. With continuous loading, bchl a

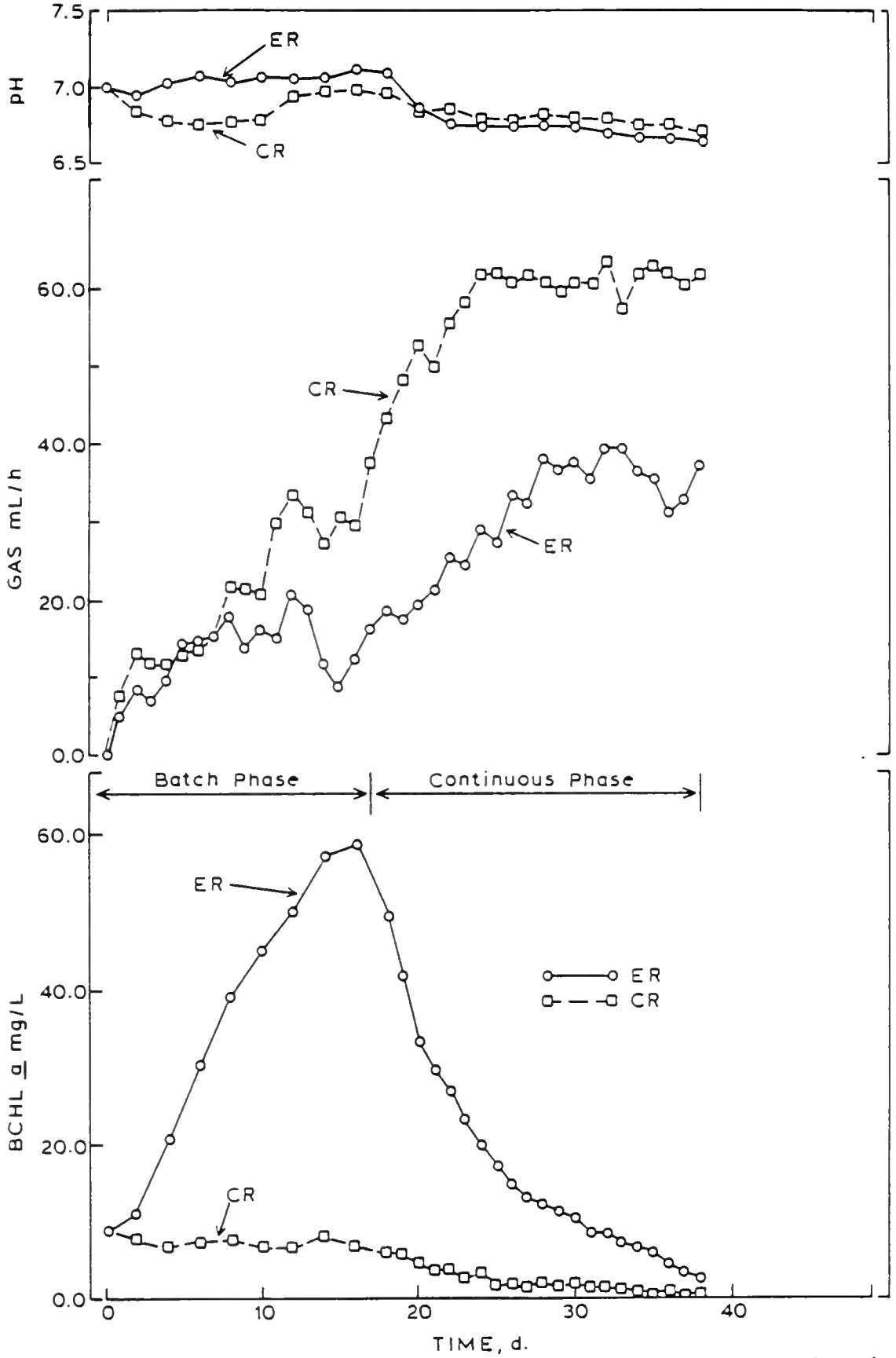

Figure 4-2. Temporal variation of bchl a, biogas production and pH during the 7-d SRT trial, series 1.

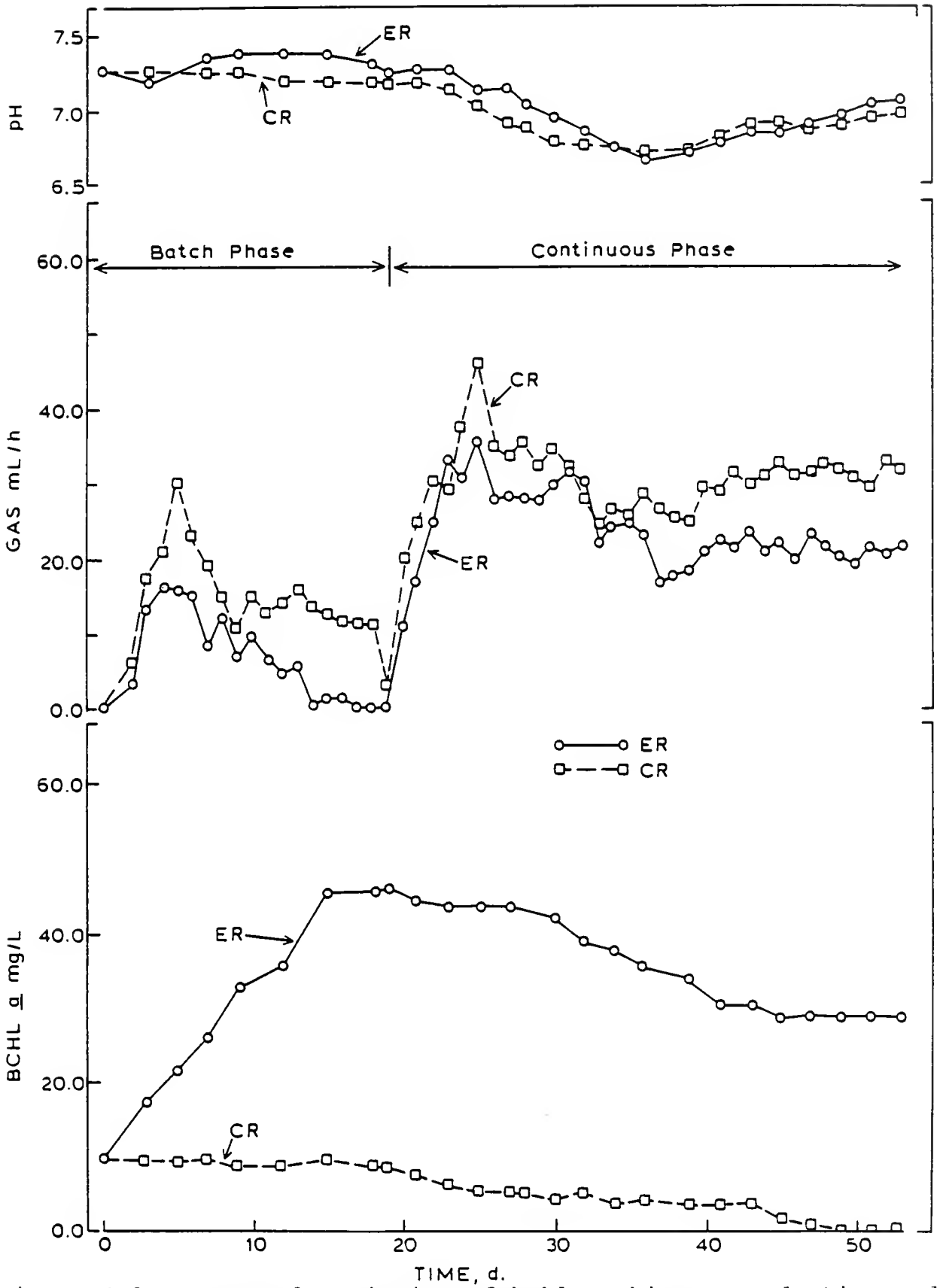

Figure 4-3. Temporal variation of bchl a, biogas production and pH during the 10-d SRT trial, series 1.

declined to a steady state value of 28.5 mg/L. In the CR, bchl a remained relatively stable at the inoculum level during the batch phase but subsequently declined to a value of 0.6 mg/L after continuous loading was begun.

Gas production was relatively low in each reactor during the batch phase. Following respective peaks of 16.6 mL/h and 25 mL/h, production in the ER and CR declined to 0.0 and 3.5 mL/h, respectively, at the end of the batch phase. On commencement of continuous loading, rapid gas evolution was observed in both reactors, and respective production peaks of 35.5 mL/h and 46.0 mL/h were attained in the ER and CR. Steady state gas production was 21.0 mL/h in the ER and 36.3 mL/h in the CR. pH ranged 7.4 to 6.6 in the ER and from 7.2 to 6.7 in the CR.

In the second trial at this SRT, no control reactor was operated. Bchl a increased from the inoculum level of 4.8 mg/L to a batch phase peak of 36.8 mg/L at day 12 (Figure 4-4). Following an initial period of decline at the start of continuous loading, bchl a increased further to a peak of 40.5 mg/L at day 31, eventually stabilizing at a steady state value of 35.5 mg/L. ^{p}H declined from 7.4 at the start of the trial to 6.7 at the end. There was no gas production during this trial.

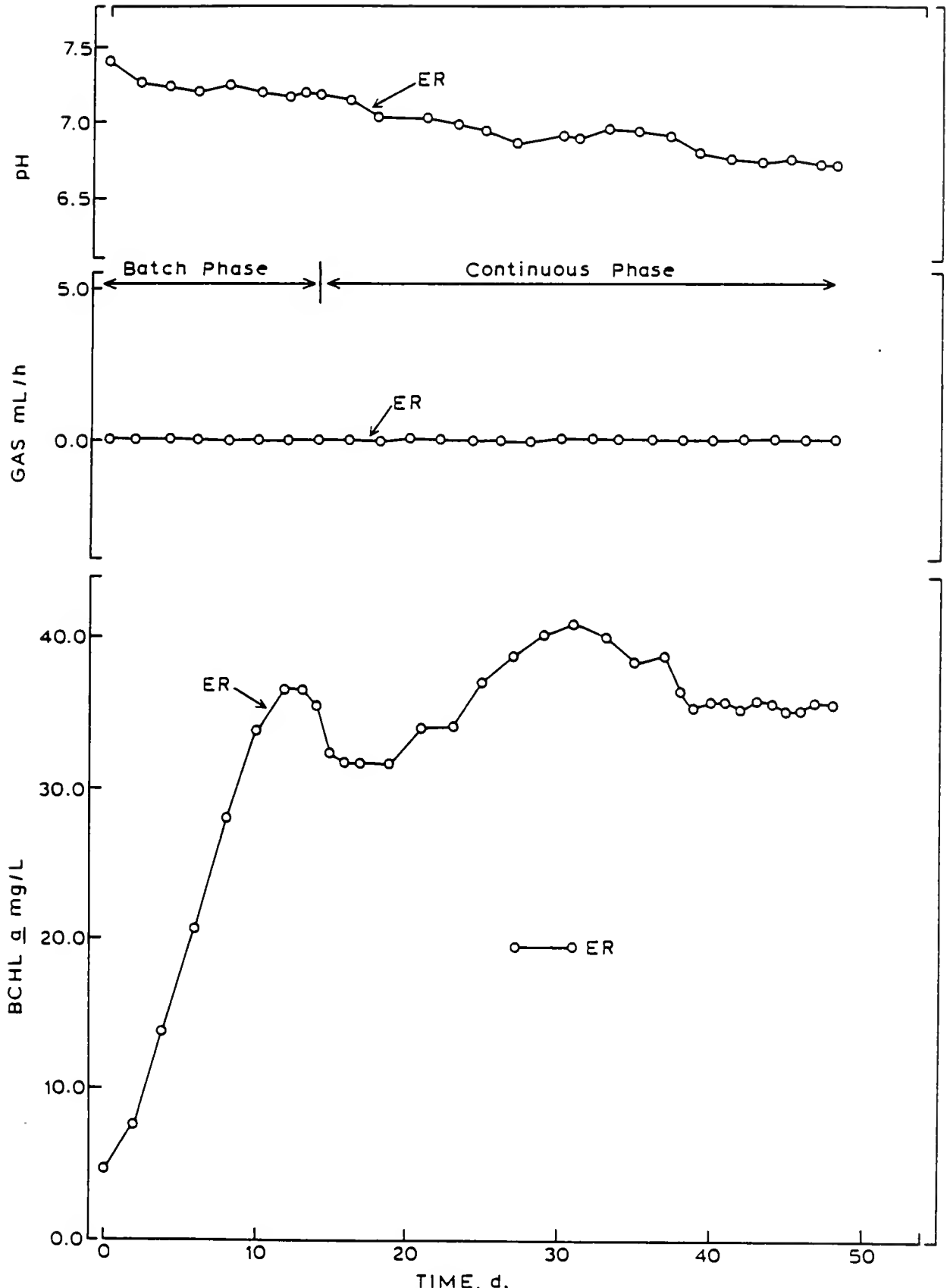

Figure 4-4. Temporal variation of bchl a, biogas production and pH during the 10-d SRT trial, series 2.

4.2.2.4 15-d SRT

The temporal variation of bchl a, gas production and pH during the 15-d SRT trial (Series 1) is shown in Figure 4-5. Bchl a in the ER increased from an inoculum level of 13.5 mg/L to a peak of 43.6 mg/L after 13 days of batch operation. Bacterial growth continued with the commencement of continuous loading, attaining a peak of 52.8 mg/L which proved to be the steady state concentration. In the CR, bchl a remained near the inoculum level during the batch phase. On commencement of continuous loading, bchl a decreased gradually to 1.2 mg/L.

Peak gas production rates of 26.0 mL/h and 30.0 mL/h were attained in the ER and CR, respectively, during the batch phase. During the continuous phase, the corresponding peak values were 44.2 mL/h and 51.0 mL/h. Steady state values were 40.0 mL/h and 47.0 mL/h, respectively. The pH in both reactors declined from an initial value of 7.5 to a steady state value of 7.0.

4.2.2.5 20-d SRT

Two experimental trials involving both batch and continuous phases were conducted at the 20-d SRT. During the batch mode of the first trial, bchl a increased to a maximum of 43.2 mg/L in the ER and 5.5 mg/L in the CR (Figure 4-6). The phototrophic inoculum level was 4.2 mg bchl a/L. Bacterial growth in the ER continued with

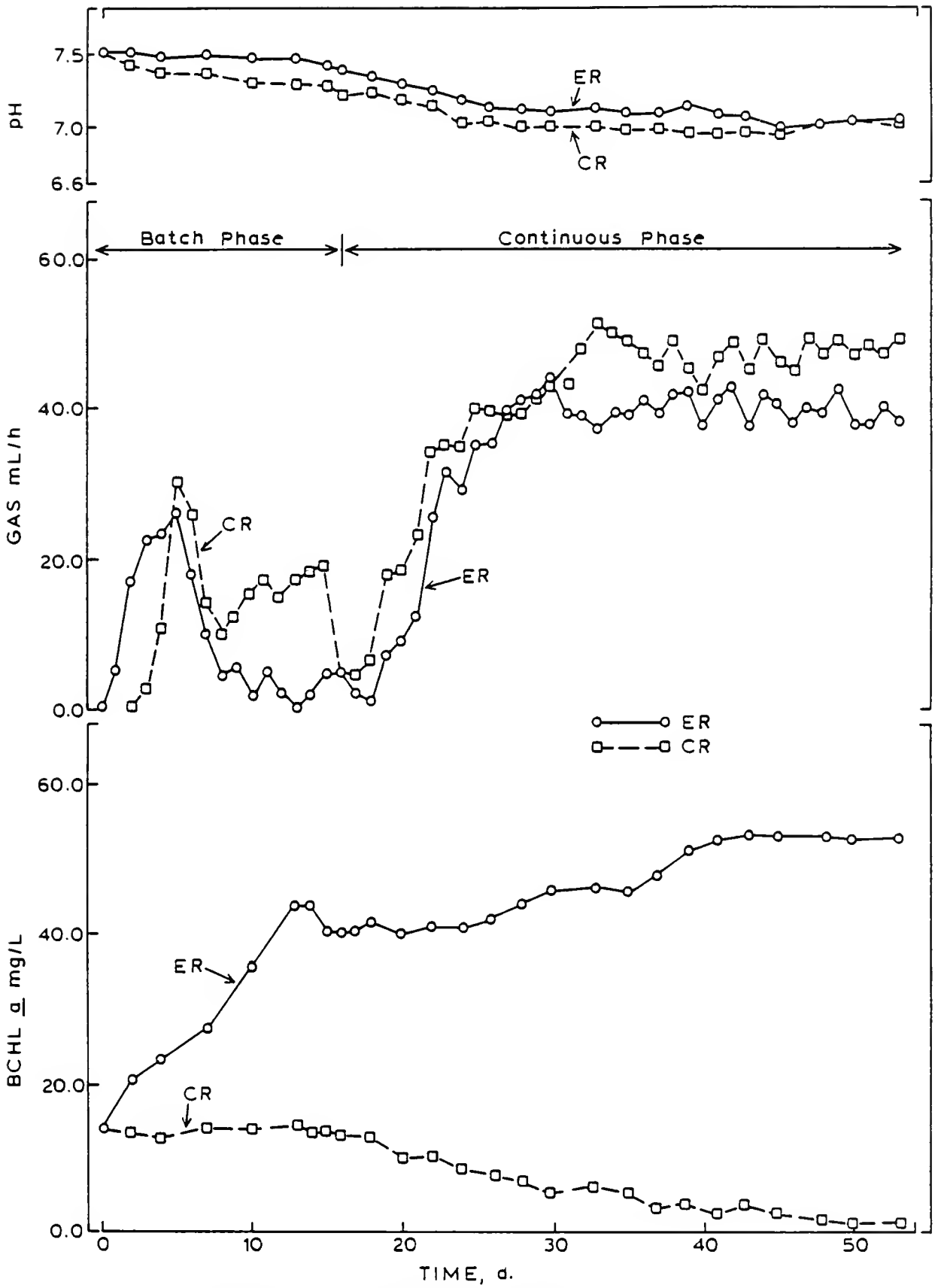

Figure 4-5. Temporal variation of bchl a, biogas production and pH during the 15-d SRT, series 1.

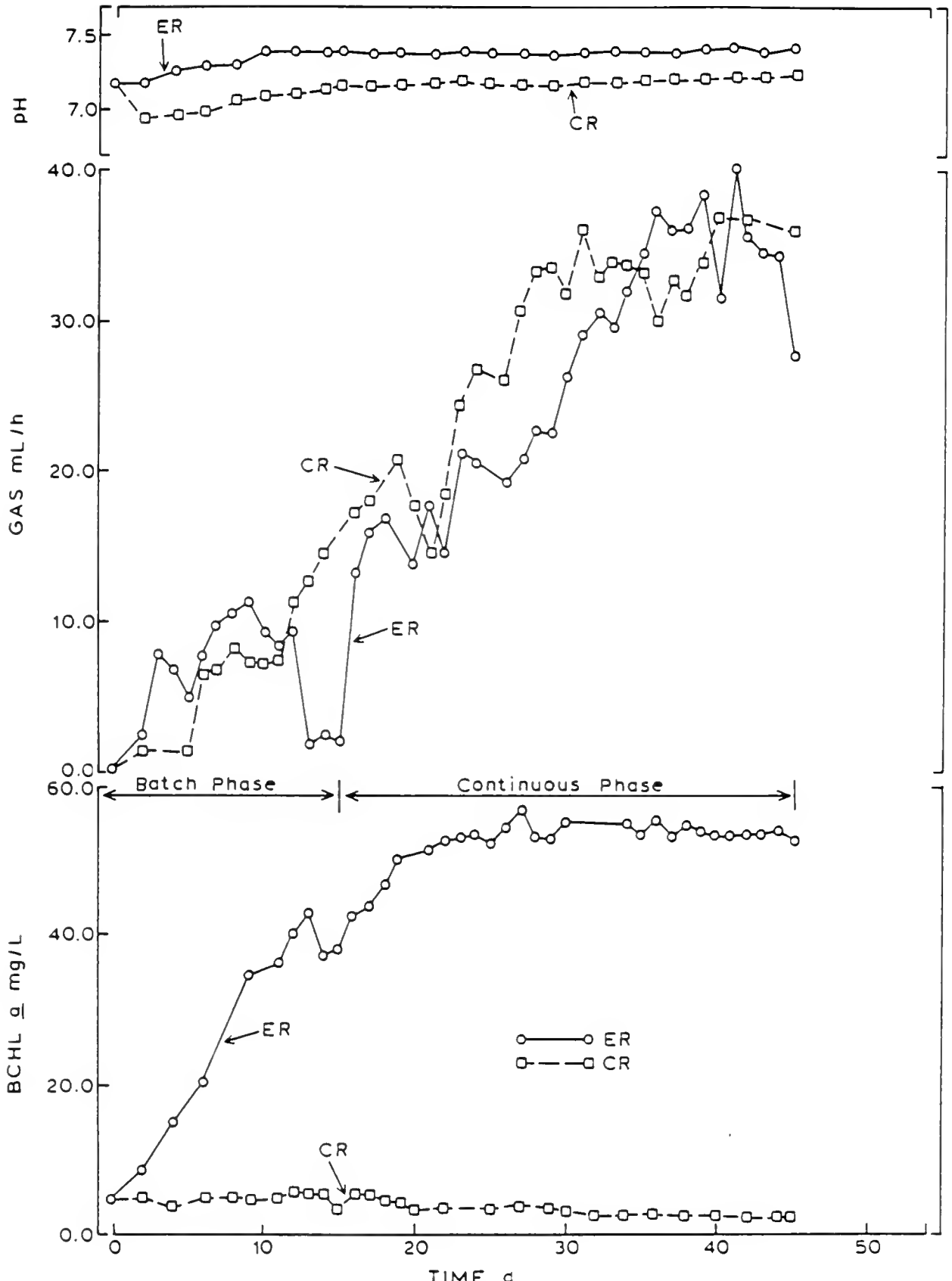

Figure 4-6. Temporal variation of bchl a, biogas production and pH during the 20-d SRT trial, series 1.

commencement of daily loading, attaining a peak value of 56.2 mg/L, before stabilizing at 53.5 mg/L. Bchl a concentration in the CR declined during the continuous loading phase to a value of 2.1 mg/L.

pH values in the ER and CR followed almost similar trends during the first 20-d SRT trial. In the ER, pH increased gradually fron an initial value of 7.2 to a final value of 7.4. In the CR, pH declined initially from 7.2 to 6.9, then increased to a final value of 7.2. Gas production increased steadily during both the batch and continuous phases. Peak rates of 41.5 mL/h and 36.5 mL/h were attained in the ER and CR, respectively.

In the second trial conducted at 20-d SRT, no control reactor was monitored (Figure 4-7). From an inoculum of 4.8 mg/L, bchl a concentration increased to a peak of 36.5 mg/L during the batch phase. After several days of continuous loading, a further increase of bchl a was noted. The steady state bchl a concentration was 45.3 mg/L. No gas production was detected during this trial. The pH was relatively steady during this experiment, averaging 7.2 over the period of steady state analyses.

4.2.2.6. 30-d SRT

Results of the 30-d SRT, Series 1 trial are plotted in Figure 4-8. During the batch phase, which lasted for 18 days, bchl a peaked at 61.1 mg/L in the ER while declining

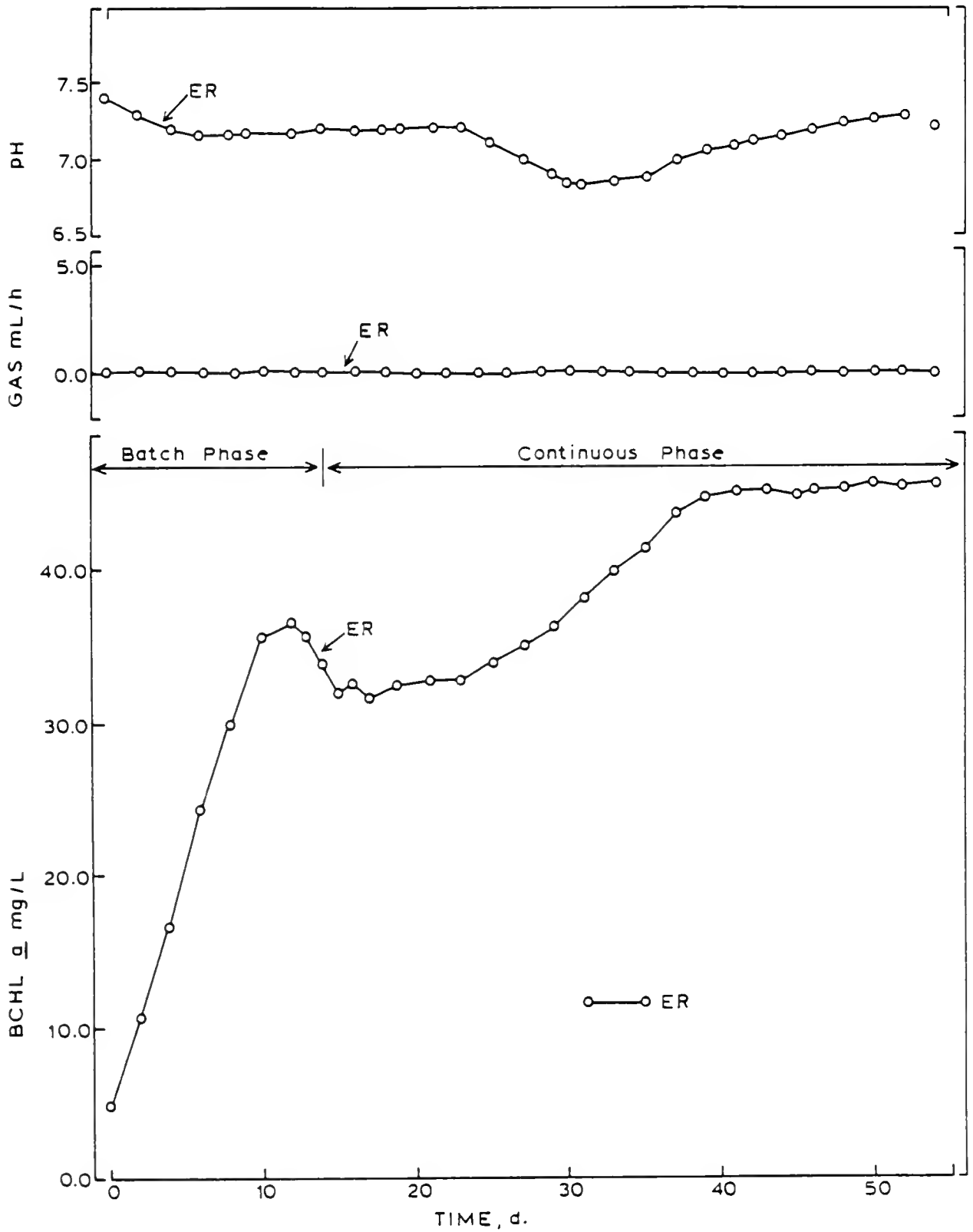

Figure 4-7. Temporal variation of bchl a, biogas production and pH during the 20-d SRT trial, series 2.

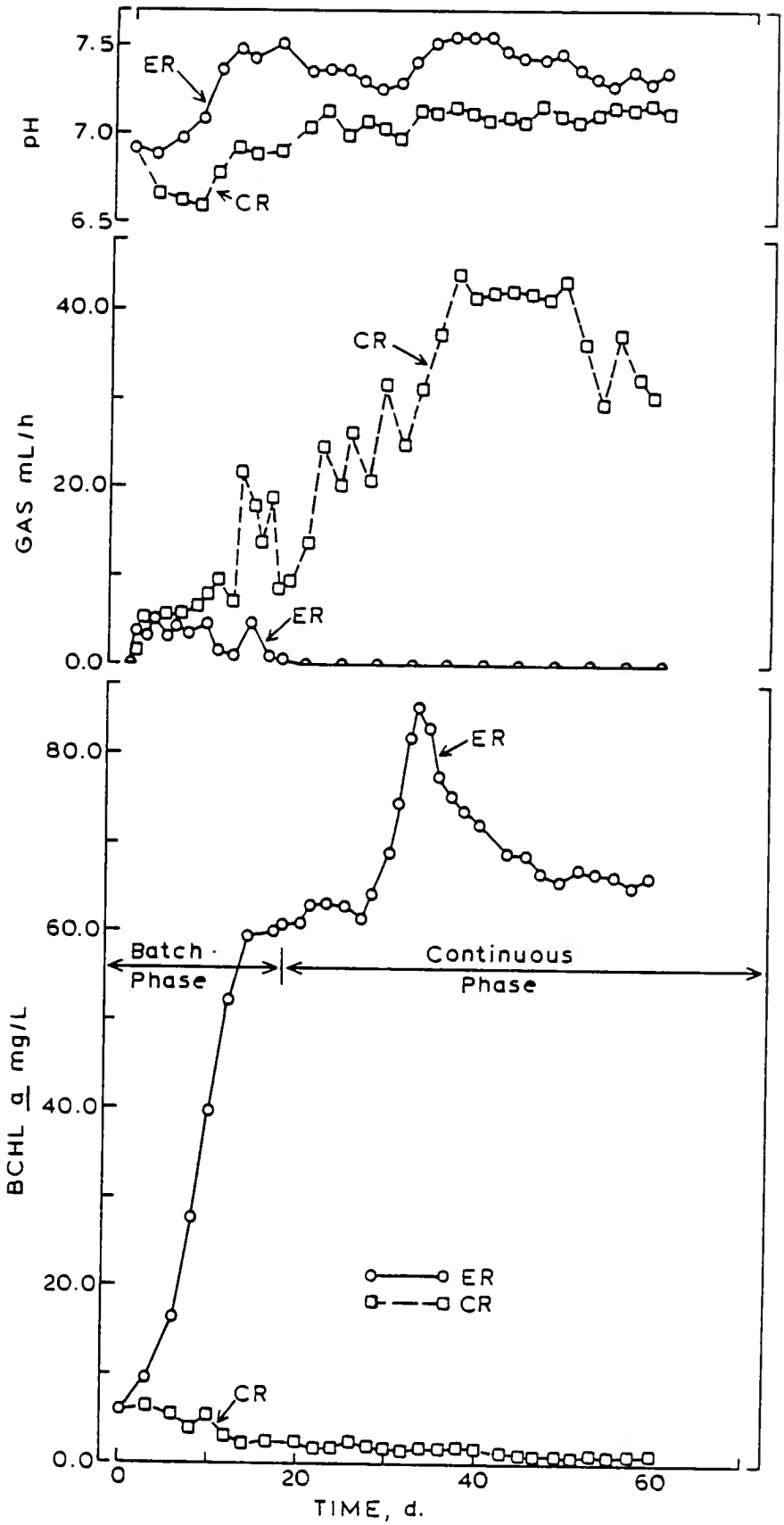

Figure 4-8. Temporal variation of bchl a, biogas production and pH during the 30-d SRT trial, series 1.

to 2.4 mg/L in the CR. Inoculum concentration was 5.8 mg bchl a/L. During the batch phase, pH increased to 7.5 in the ER, and declined 6.6 in the CR. Initial pH was 6.9. Gas production in the ER was poor, peaking at 5.2 mL/h and declining subsequently to zero after 17 days of batch operation. Gas production in the CR peaked at 22 mL/h then declined to 8.5 mL/h at the end of the phase.

In the continuous phase bchl a in the ER peaked at 85.1 mg/L, then declined to a steady state bchl a concentration of 66 mg/L. In the CR bchl a fell gradually to 0.6 mg/L by the end of the trial. After an initial climbing trend, pH was relatively steady, averaging 7.3 in the ER and 7.1 in the CR over the period of steady state analyses.

There was no gas production in the ER during the continuous phase of this trial. In the CR, gas production peaked at 46.5 mL/h and declined subsequently to 29.5 mL/h.

4.2.3. Continuous/Continuous Mode Trials

4.2.3.1. 8.5-d SRT

Evaluation of bacterial response to continuous loading at this residence time was commenced from the steady state conditions which existed at the end of the 10-d SRT trial in the second series of experiments. After the loading rate was increased, bchl a declined from the 10-d steady state value of 34.5 mg/L to 2.5 mg/L over a period of 33 days

(Figure 4-9). pH fell from 6.5 to 5.9 during this period. No gas production was observed.

4.2.3.2. 15-d SRT

In the second trial at 15-d SRT, continuous loading commenced at the end of the 20-d SRT (Series 2) trial. After the loading rate was increased bchl *a* decreased from an initial level of 45.5 mg/L to a steady state value of 38.8 mg/L (Figure 4-10). pH stabilized at an average of 6.9 after starting at 7.2. No gas production was evident during this trial.

4.2.3.3. 30-d SRT

The 30-d Series 2 trial followed at the end of the 15-d Series 2 trial. Bchl *a* was 38.8 mg/L and pH was 6.9 before the loading rate was changed. After the decrease in loading rate, bchl *a* increased to a value of 60.5 mg/L (Figure 4-11). pH fluctuated somewhat, eventually stabilizing at 6.7. No gas production was observed during this trial.

4.3 Growth Characteristics of Phototrophic Bacteria

4.3.1. Batch Growth Characteristics

In trials having a batch mode, bchl *a* at first increased exponentially with time. The rate constant characterizing initial growth was determined by fitting an

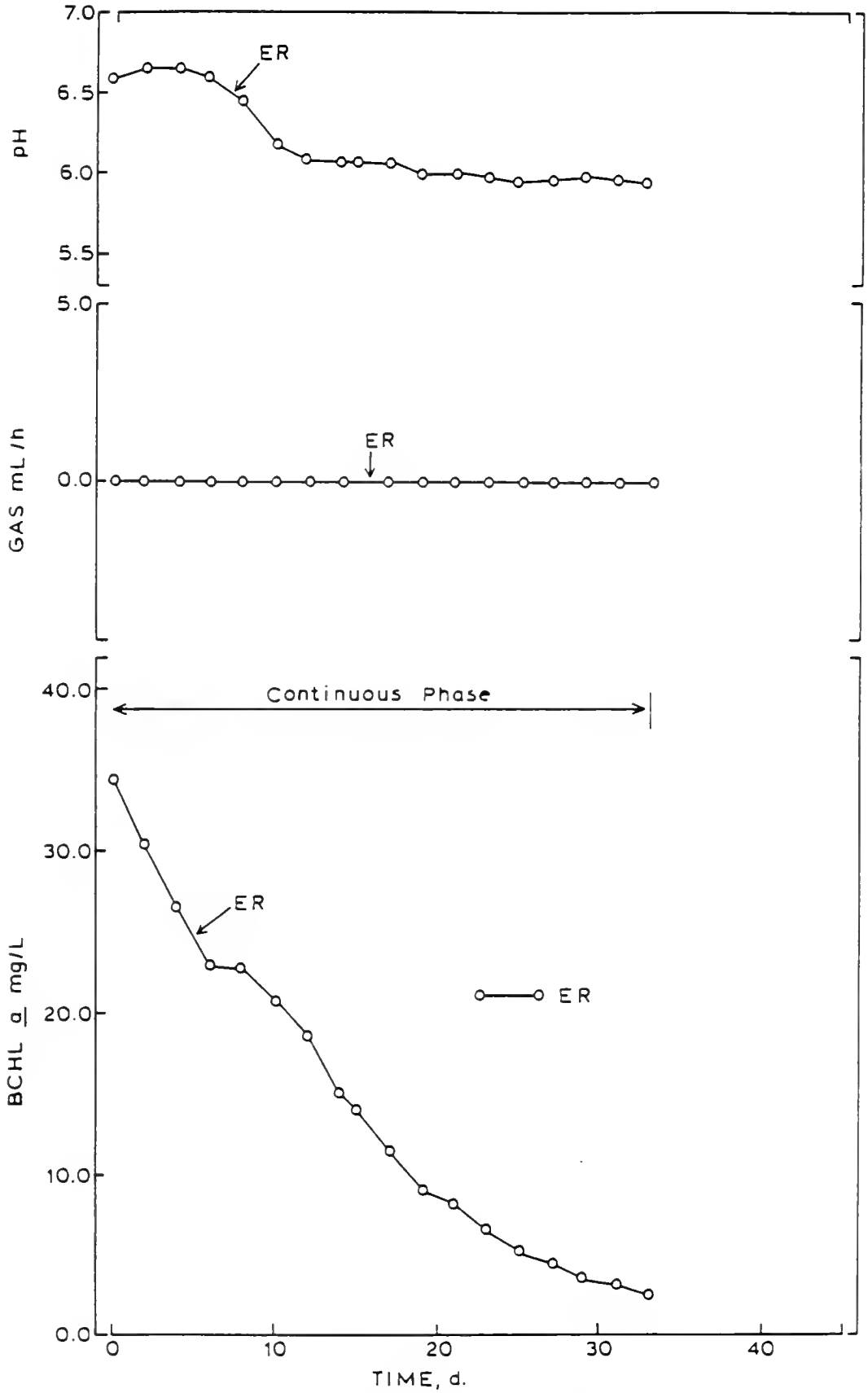

Figure 4-9. Temporal variation of bchl a, biogas production and pH during the 8.5-d SRT trial, series 2.

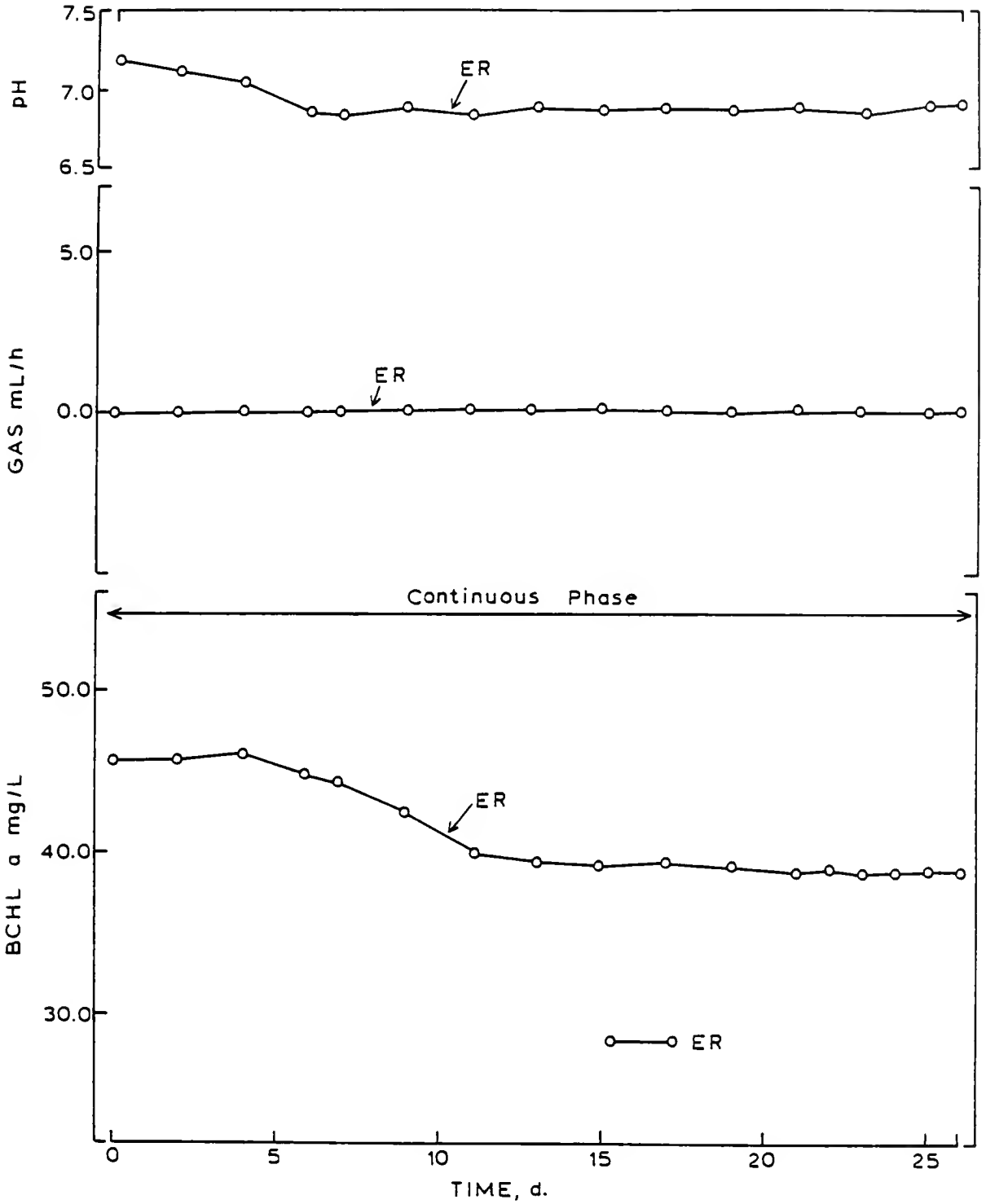

Figure 4-10. Temporal variation of bchl a, biogas production and pH during the 15-d SRT trial, series 2.

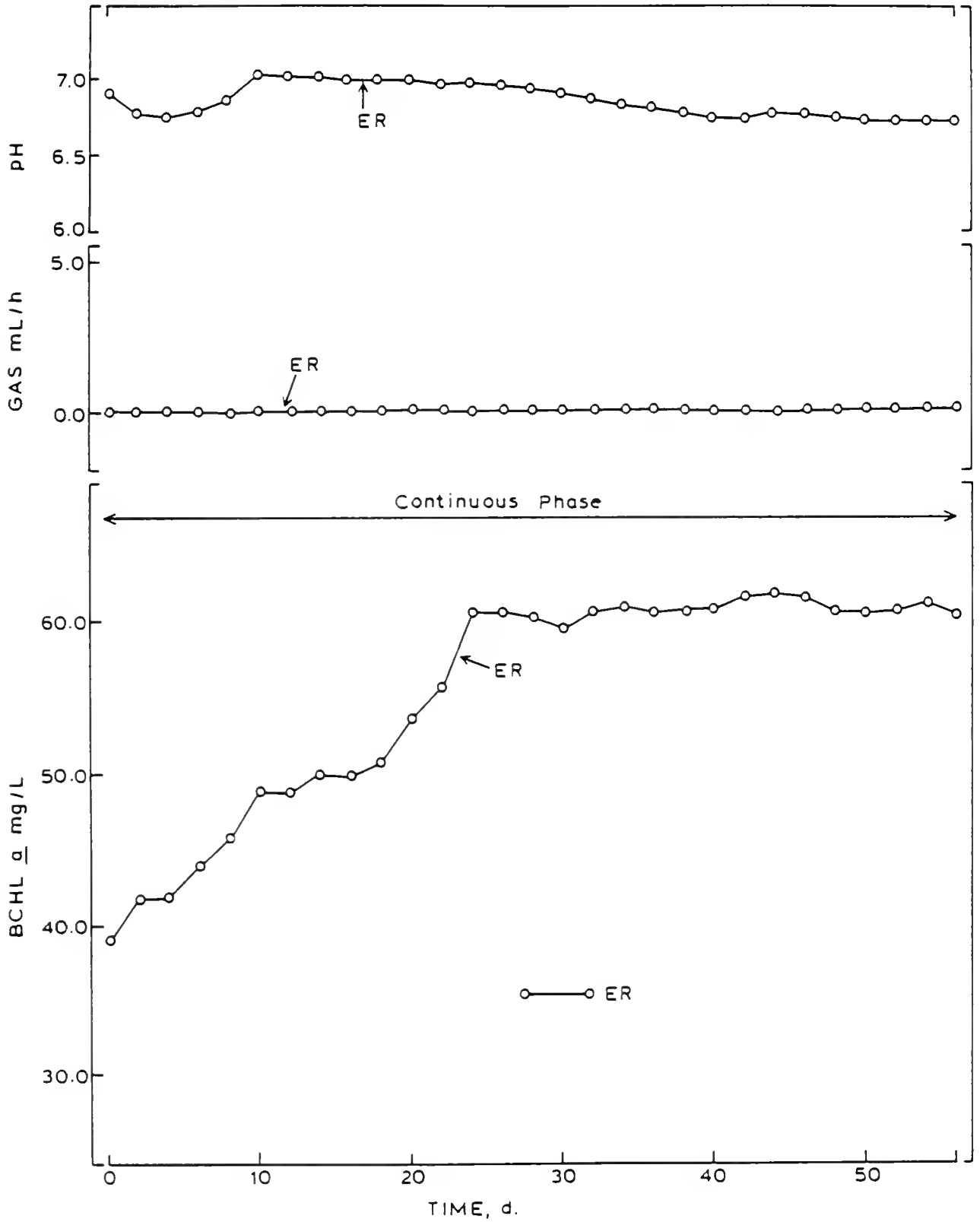

Figure 4-11. Temporal variation of bchl a, biogas production and pH during the 30-d SRT trial, series 2.

exponential relationship to the initial growth data. This relationship had the form;

$$y = y_o e^{\mu_m t} \qquad (4\text{-}1)$$

where t = time (days), y = bchl *a* concentration (mg/L) at time t, y_o = bchl *a* concentration at time t_o, and μ_m = rate constant characterizing initial (exponential) growth. The parameter μ_m is an estimate of the maximum specific growth rate of the phototrophic bacteria under conditions (illumination, waste composition, temperature, pH). Estimates of maximum specific growth rate are given in Table 4-1. Values tended to fall either in a lower range (0.04-0.05 d^{-1}) or a higher range (0.10-0.12 d^{-1}). The theoretical washout SRT for the higher range of $_m$ would be equal to 1/0.11 d^{-1} or 9.1 days. The overall average for μ_m was 0.083 d^{-1}, which corresponds to a theoretical washout SRT of 12.0 days.

4.3.2. Steady State Growth Kinetics

Steady state biomass concentrations measured in terms of bchl *a* and protein, total and volatile solids, and suspended solids are plotted versus solids retention time in Figures 4-12 and 4-13 and 4-14 respectively. Bchl *a* in the ER, averaged over the two trials, increased from 32 mg/L to 63 mg/L for detention periods 10 to 30 days and showed a

Table 4-1. Batch growth characteristics of phototrophic sulfur bacteria cultured in swine waste medium.

SRT	Series	μ_{max} day^{-1}	pH range	Bchl *a* range mg/L
5	1	0.05	6.8 - 7.2	16.3 - 59.0
7	1	0.11	6.9 - 7.1	8.5 - 59.3
10	1	0.05	7.2 - 7.4	9.7 - 45.7
	2	0.11	7.1 - 7.4	4.8 - 36.4
15	1	0.04	7.4 - 7.5	13.6 - 39.9
20	1	0.10	7.2 - 7.4	4.2 - 38.4
30	1	0.12	7.2 - 7.4	1.0 - 61.1

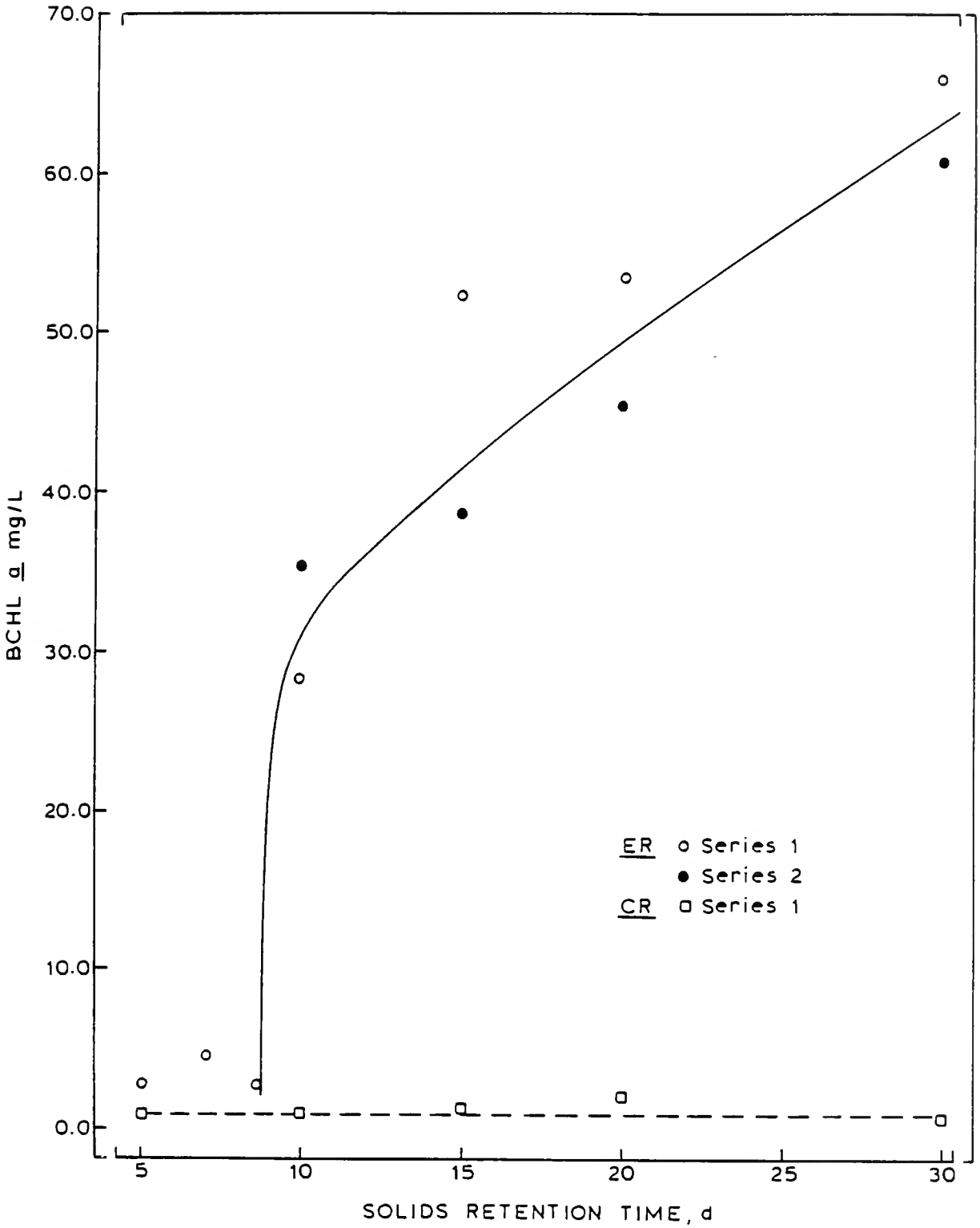

Figure 4-12. Relationship of bchl a to solids retention time.

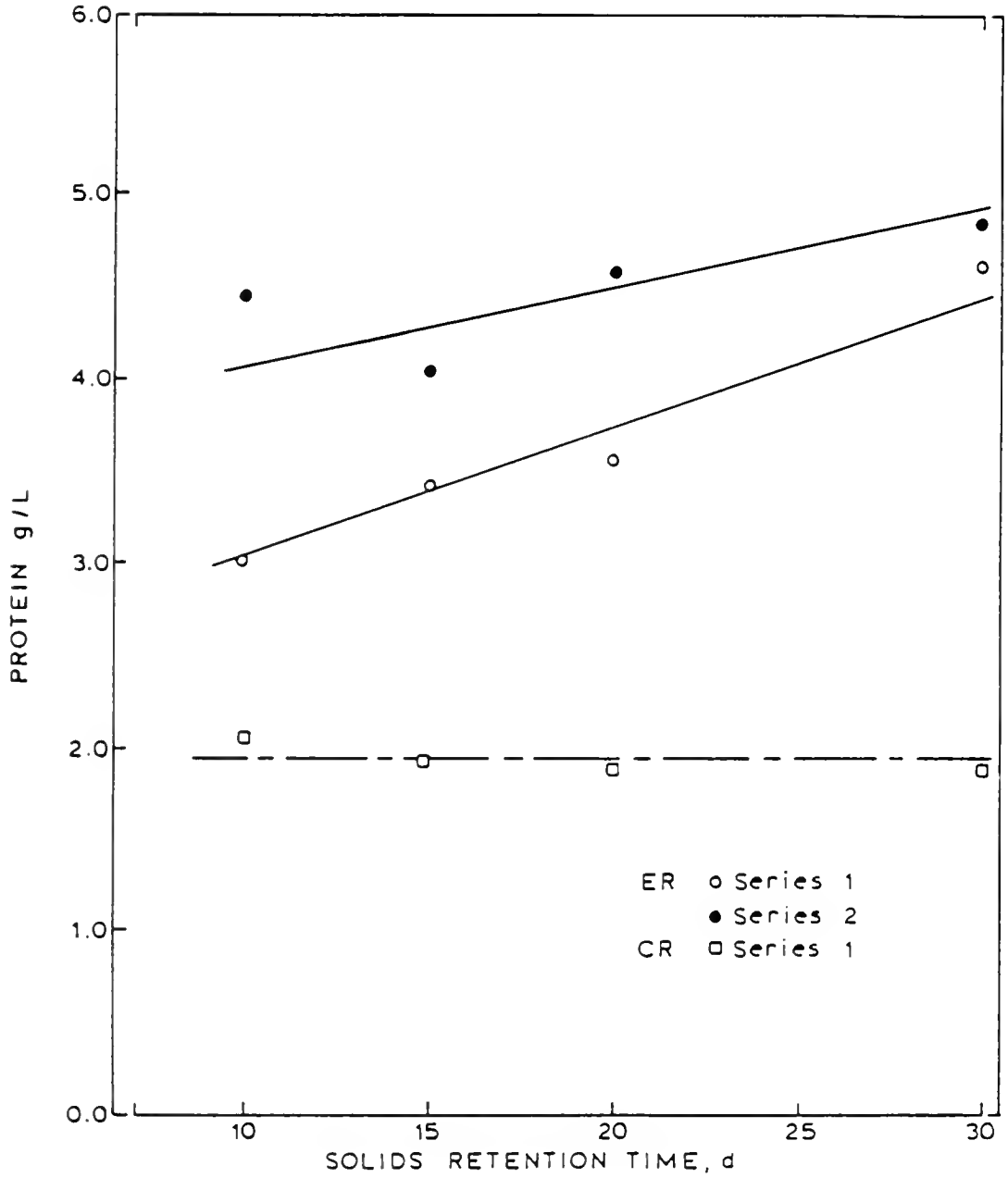

Figure 4-13. Relationship of protein to solids retention time.

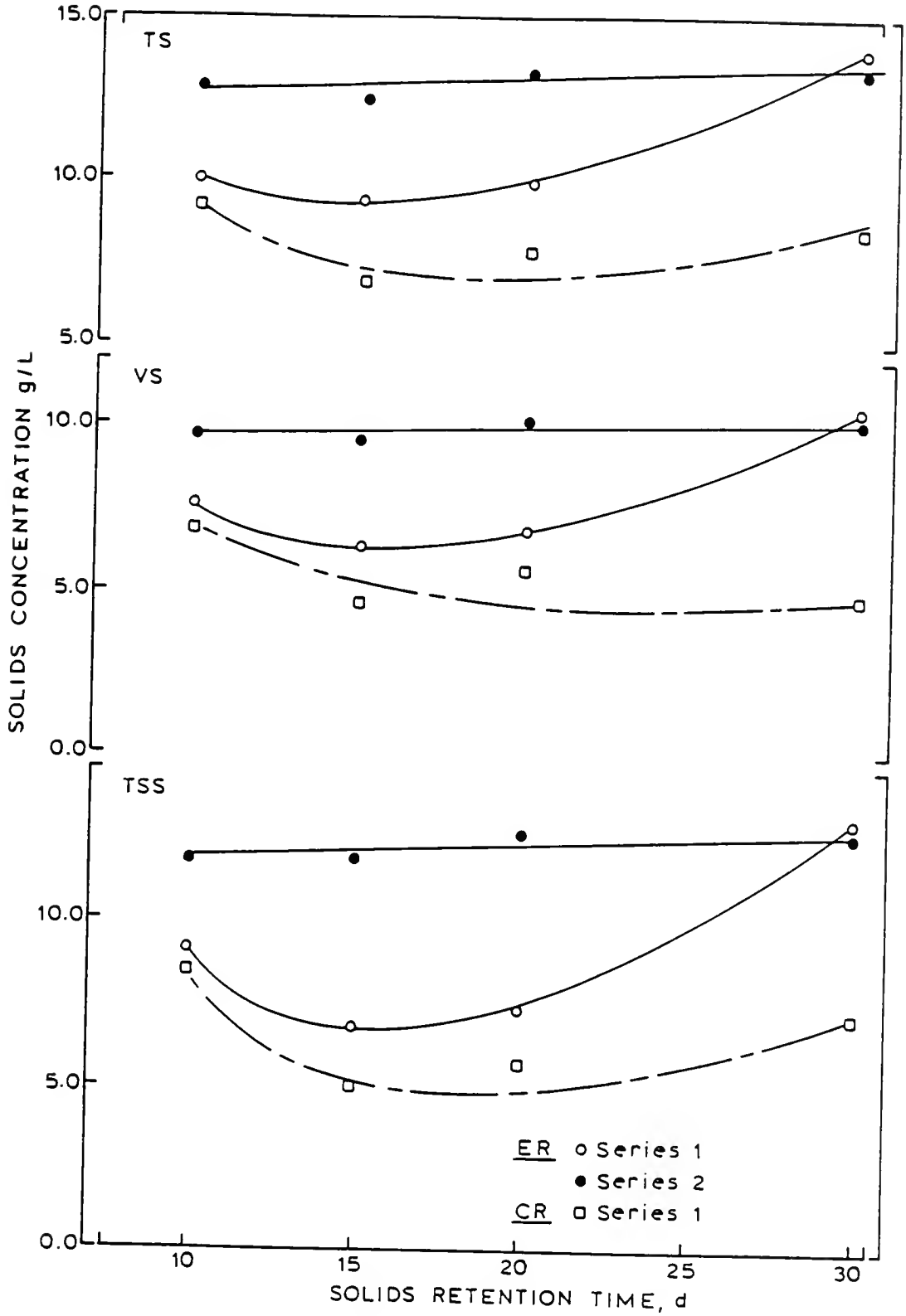

Figure 4-14. Relationship of solids concentration to solids retention time.

sharp decline below a detention time of 10 days (Figure 4-12). With washout occurring at the 8.5 days SRT trial, the minimum detention time lies between 8.5 and 10 days. Bchl a in the CR was nearly zero over the range of SRTs investigated. Protein in the ER increased significantly with retention time in both series of experiments. In Series 1, protein increased from 3.0 g/L at 10 days to 4.6 g/L at 30 days. Corresponding values for Series 2 were 4.4 g/L and 4.9 g/L (Figure 4-13). In the CR this parameter remained constant at approximately 1.9 mg/L.

Total, volatile and suspended solids variations all indicated similar response patterns to varying SRTs (Figure 4-14). During the first trial, TS in the ER, declined initially from 10 g/L at 10 days to 8.4 g/L at 15 days, followed by an increase to a maximum value of 13.9 g/L at 30 days. Likewise, VS decreased initially from 7.1 g/L at 10 days, to 6.3 g/L at 15 days and then increased to 10.5 g/L at 30 days. The corresponding TSS values were 8.4, 6.2, and 13.1 g/L at 10, 15, and 30 days, respectively. In the CR, the pattern of behavior was similar, with the exception that the maximum values for all parameters occurred at the minimum SRT. Greatest divergence between these parameters in the ER and CR occurred at 30-d SRT where differences of 5.5, 5.7, and 6.1 g/L in TS, VS, and TSS concentrations, respectively, were observed. Variation of solids concentration in the ER with SRT was not significant

in the Series 2 experiments. TS concentration varied from 11.8 g/L at 10 days to 13.3 g/L at 30 days. Correspondingly, VS ranged 9.7 to 10.2 g/L, and TSS ranged 11.8 to 12.6 g/L.

4.3.3. Biomass Productivity

Productivity expressed in terms of bchl a and protein, total and volatile solids, and suspended solids is plotted versus dilution rate in Figures 4-15, 4-16 and 4-17, respectively. In Series 1 experiments, bchl a productivity in the ER increased from 7.7 mg/d (2.2 mg/L-d) at a dilution rate of 0.033 d^{-1} to a peak of 12.2 mg/d (3.5 mg/L-d) at dilution rate 0.067 d^{-1}, subsequently declining to 2.9 mg/d at dilution rate 0.10 d^{-1} (Figure 4-15). All other parameters showed increased productivity from the lowest to the highest dilution rate during Series 1. In the ER, protein increased from 0.5 g/d (0.14g/L-d) to 1.0 g/d (0.29 g/L-d), TS productivity ranged 1.6 to 3.5 g/d (0.46 to 1.0 g/L-d), VS productivity ranged 1.2 to 2.4 g/d (0.34 to 0.69 g/L-d), and TSS productivity ranged from 1.5 to 2.9 g/d (0.43 to 0.83 g/L-d). In the CRs only bchl a showed no increase in productivity with increased dilution rate. Productivity was generally less in the CR than in the ER, the greatest differences being observed at the lower dilution rates. At the higher dilution rates, TS, VS and TSS productivities in the ER and the CR are close.

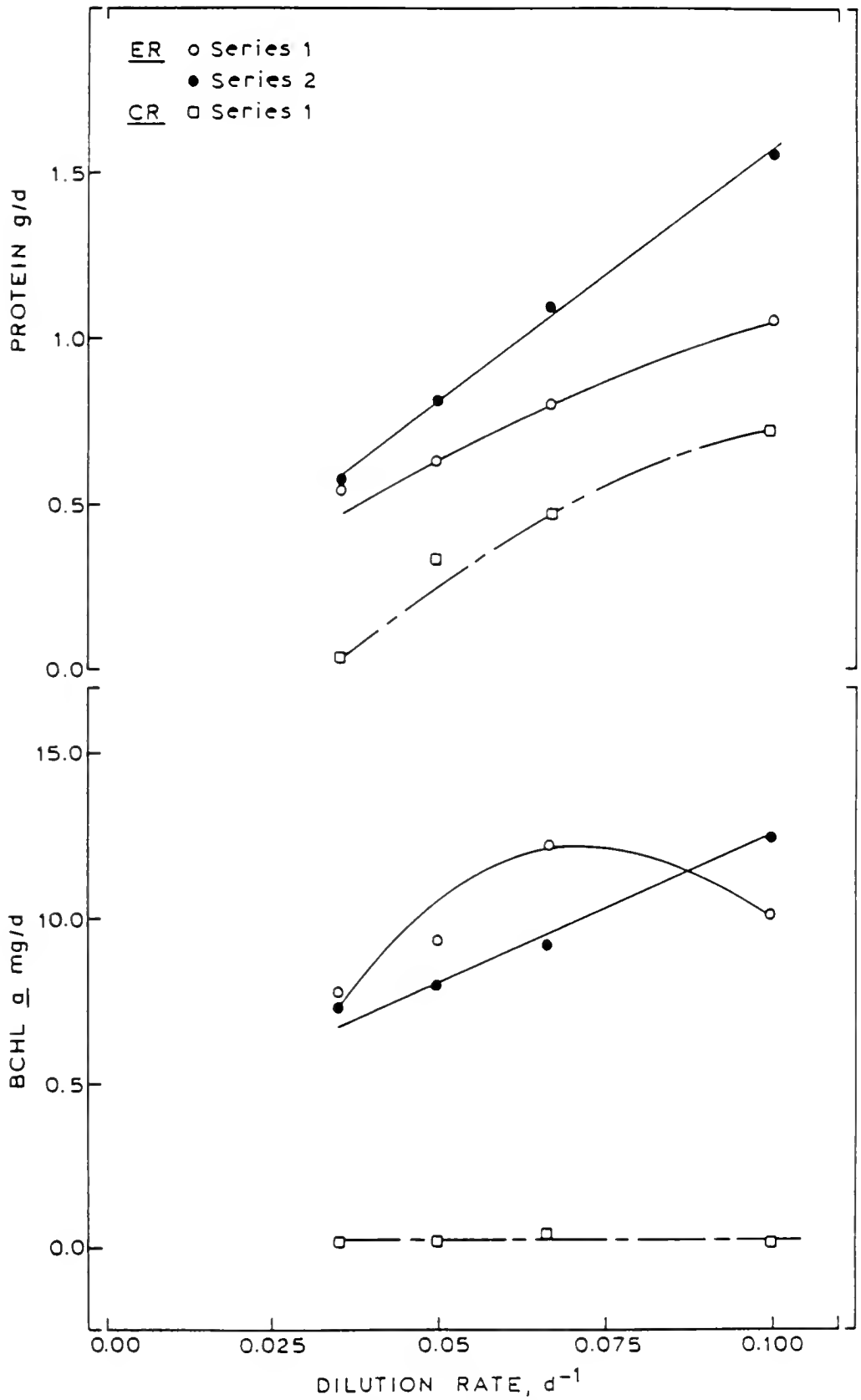

Figure 4-15. Relationship of productivity in terms of bchl a and protein to dilution rate.

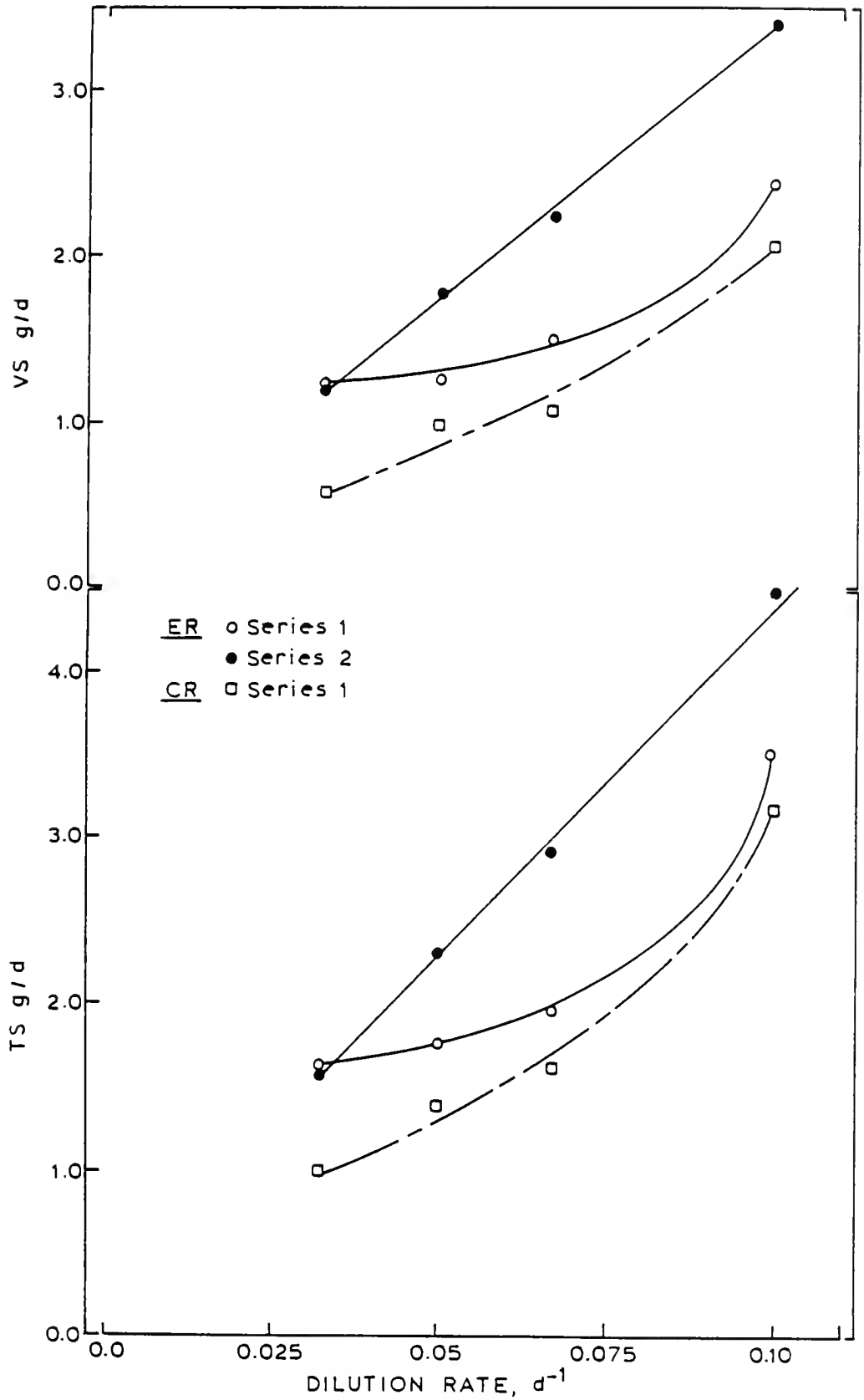

Figure 4-16. Relationship of productivity in terms of total solids and volatile solids to dilution rate.

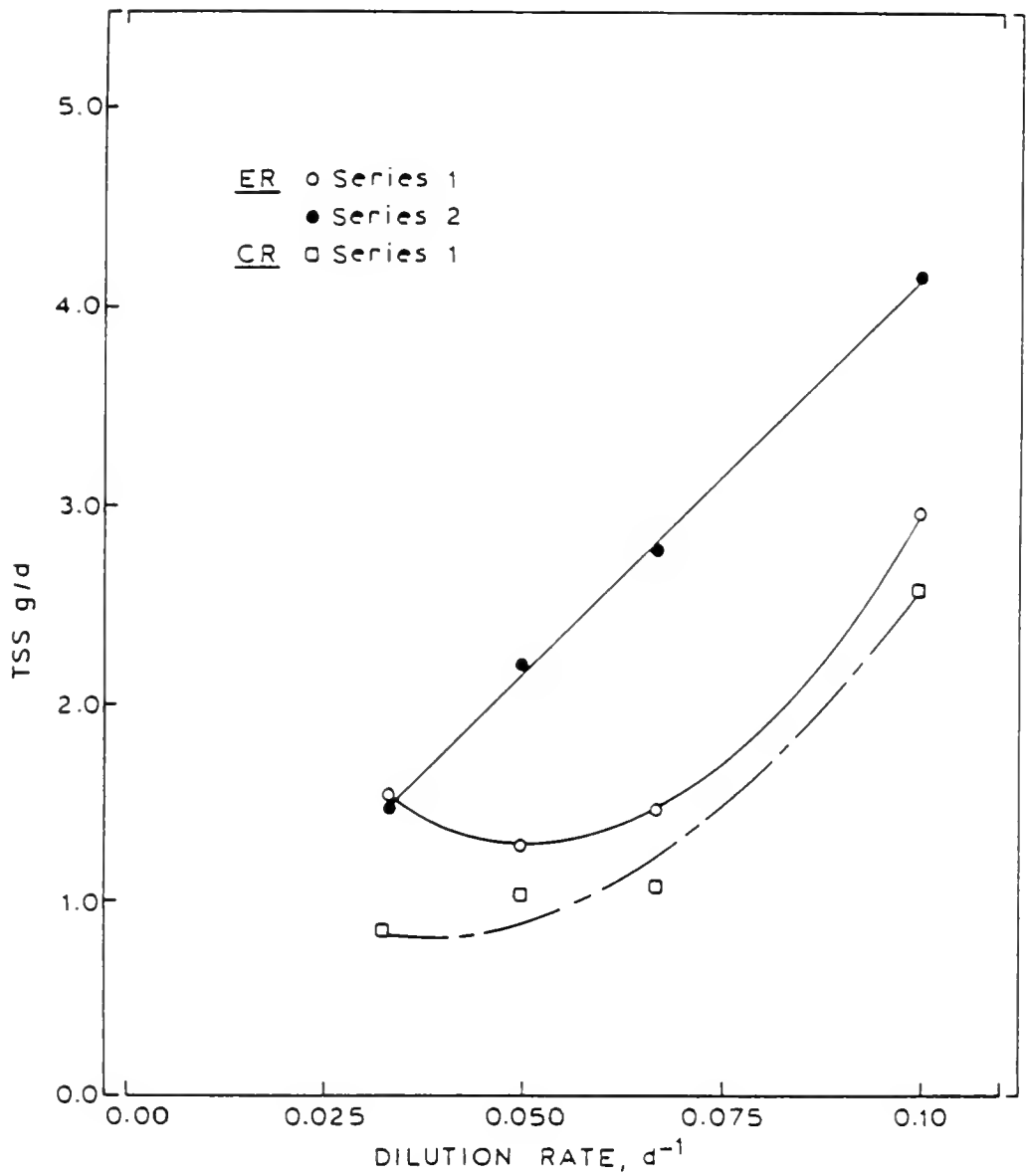

Figure 4-17. Relationship of productivity in terms of total suspended solids to dilution rate.

In Series 2 experiments, productivity in the ER increased for all parameters from the lowest dilution rate to the highest. Bchl *a* productivity increased from 7.10 mg/d (2.03 mg/L-d) at dilution rate 0.033 d^{-1} to 10.02 mg/d (2.86 mg/L-d) at dilution rate 0.10 d^{-1}. Corresponding ranges for protein, TS, VS and TSS were 0.57 to 1.55 g/d (0.16 to 0.44 g/L-d), 1.56 to 4.47 g/d (0.45 to 1.28 g/l-d), 1.19 to 3.40 g/d (0.34 to 0.97 g/L-d) and 1.47 to 4.14 g/d (0.42 to 1.18 g/L-d), respectively.

4.4. Waste Conversion

4.4.1. Gas Production and Quality.

Plots of steady state gas production and quality are shown in Figure 4-18 for both the ER and CR. All values were adjusted to a pressure of 1 atmosphere and temperature 0°C. Gas production in the CR was greater than that in the ER at every SRT used, although the volume of methane produced in each reactor (Figure 4-19) was very nearly the same. There is similarity between both curves, each showing a peak at about 15-d SRT. Peak gas production values were 1.07 L/d (0.30 L/L-d) in the CR and 0.90 L/d (0.26 L/L-d) in the ER, equivalent to 0.63 L/d (0.18 L/L-d) and 0.61 L/d (0.17 L/L-d) methane, respectively. Gas production in the ER declined to zero at 30 days, while that in the CR levelled off to a rate of 0.65 L/d (0.19 L/L-d). Methane content of the reactor gas increased with retention time for

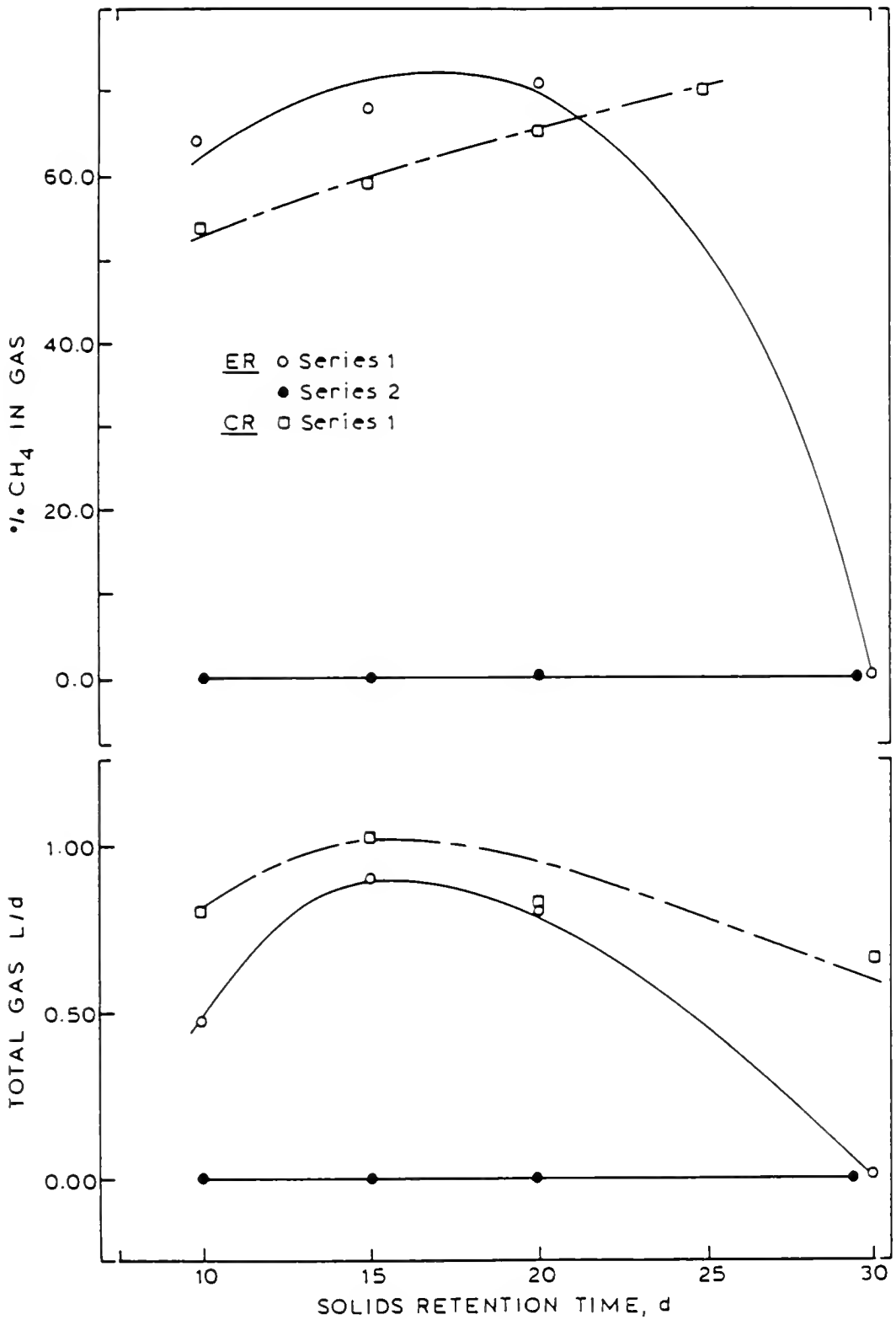

Figure 4-18. Effect of solids retention time on gas production and quality.

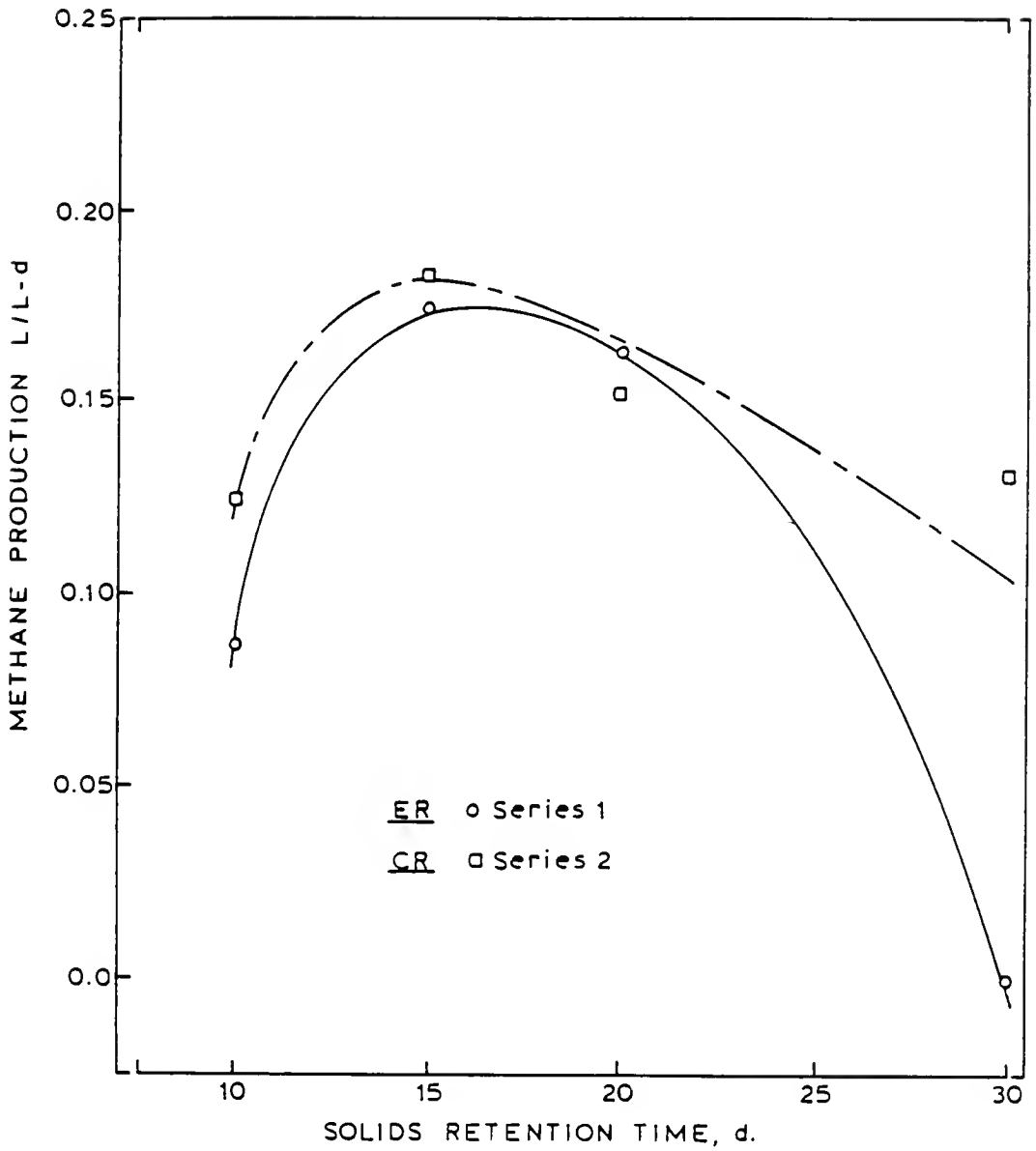

Figure 4-19. Effect of solids retention time on methane production.

both reactors, ranging 64-71 percent in the ER and 53-70 percent in the CR. The peak value of 71 percent occurred in the ER at 20 days. Percentages of CO_2 showed only small variation over the range of detention times, lying between 22 and 30 percent in the CR and between 20 and 21 percent in the ER.

Total gas production related to soluble COD destroyed is tabulated in Table 4-2. For the ER the values ranged from 0.08-0.29 L gas/g COD destroyed, and in the CR, the corresponding values were 0.18-0.40 L gas/g COD destroyed, corresponding to 0.05-0.21 and 0.10-0.28 L methane/g COD destroyed, respectively.

Methane production related to COD and VS loading is indicated in Table 4-3. For COD loading which decreased from 6.7 g/d at 10-d SRT to 2.3 g/d at 30-d SRT, methane production in the ER increased from 0.04 L/g COD added (0.01 L/L-g) at 10 days to 0.18 L/g COD added (0.05 L/L-g) at 20 days reducing to zero at 30 days. In the CR, the values ranged from 0.06 L/g COD added (0.02 L/L-g) at 10 days, to 0.20 L/g COD added (0.06 L/L-g) at 30 days. VS loading ranged from 3.2 g/d at 10 days to 1.2 g/d at 30 days. Methane production in the ER was 0.09 L/g VS added (0.03 L/L-g) at 10 days increasing to o.36 L/g VS added (0.10 L/L-g) at 20 days, and reducing to zero at 30 days. In the CR, methane production values were 0.14 L/g VS added (0.04

Table 4-2. Steady state gas production at STP related to COD destroyed.

Parameter	Reactor	SRT 10	SRT 15	SRT 20	SRT 30
Flow L/d	ER	0.350	0.233	0.175	0.117
	CR	0.350	0.233	0.175	0.117
Influent COD mg/L	ER	19 165	18 022	17 988	19 683
	CR	19 165	18 022	17 988	19 683
COD loading g/d	ER	6.708	4.199	3.150	2.303
	CR	6.708	4.199	3.150	2.303
Effluent COD mg/L	ER	3 083	2 760	2 455	2 333
	CR	6 516	5 407	5 576	5 708
COD removed mg/L	ER	16 082	15 262	15 533	17 350
	CR	12 649	12 615	12 412	13 975
COD removed %	ER	84	85	86	88
	CR	66	70	69	71
COD removed g/d	ER	5.629	3.556	2.718	2.030
	CR	4.427	2.939	2.174	1.635
Total gas (STP) L/d	ER	0.462	0.898	0.795	0.000
	CR	0.802	1.066	0.813	0.651
CH_4 (STP) %	ER	64.25	67.50	71.25	0.00
	CR	53.75	59.25	64.75	69.75
Total CH_4 L/d	ER	0.297	0.606	0.566	0.000
	CR	0.431	0.632	0.526	0.454
Specific CH_4 L/L/d	ER	0.085	0.173	0.162	0.000
	CR	0.123	0.181	0.150	0.130
Total gas/COD removed L/g/d	ER	0.082	0.253	0.293	0.000
	CR	0.181	0.363	0.374	0.398
Total CH_4/gCOD removed L/g/d	ER	0.053	0.170	0.208	0.000
	CR	0.097	0.215	0.242	0.278

Table 4-3. Steady state gas production at STP* related to volatile solids and COD loading

Parameter	Reactor	SRT, days 10	15	20	30
Total gas (STP) L/d	ER	0.462	0.898	0.795	-
	CR	0.802	1.066	0.813	0.651
Total CH_4 L/d	ER	0.297	0.606	0.566	-
	CR	0.431	0.632	0.526	0.454
VS loading g/d	ER	3.205	2.243	1.584	1.176
	CR	3.205	2.243	1.584	1.176
COD loading g/d	ER	6.708	4.199	3.150	2.303
	CR	6.708	4.199	3.150	2.303
CH_4/gVS added L/g	ER	0.093	0.270	0.357	-
	CR	0.135	0.282	0.332	0.386
CH_4/gVS added L/L vol-gVS	ER	0.027	0.077	0.102	-
	CR	0.039	0.081	0.095	0.110
CH_4/gCOD added L/g	ER	0.044	0.144	0.180	-
	CR	0.064	0.151	0.167	0.197
CH_4/gCOD added L/L vol-gCOD	ER	0.013	0.041	0.051	-
	CR	0.018	0.043	0.048	0.056

* 1 atmosphere, 0°C.

L/L-g) at 10 days, increasing to 0.39 L/g VS added (0.11 L/L-g).

4.4.2. Oxygen Demand

Comparison of soluble BOD_5 and COD removals in the ER and CR at various dilution rates is given in Figure 4-20. In each reactor soluble removal rates increased with retention time. At 10 days, with influent COD level of 19200 mg/L, corresponding to a loading of 6.71 gCOD/d (1.92 gCOD/L-d), percentage removals were 84 and 66 for the ER and CR, respectively, representing the maximum total daily COD removals of 5.63 gCOD/d (1.61 gCOD/L-d) and 4.43 gCOD/d (1.26 gCOD/L-d), respectively, attained in these studies.

Steady state methane production in the ER at 10-d SRT was 0.30 L/d (0.085 L/L-d) and in the CR was 0.43 L/d (0.12 L/L-d). Since two moles of O_2 are required to oxidize one mole of methane gas, the COD equivalent of methane is 64 gCOD/mole methane (1 mole = 22.4 L STP). From this relationship the theoretical value of the COD consumed in the production of methane was computed as shown in Table 4-4. These values indicate that at the time of optimum gas production at 15-d SRT 1.8 gCOD/day were available for cell synthesis and maintenance, and 1.1 gCOD/day were available for these activities in the CR.

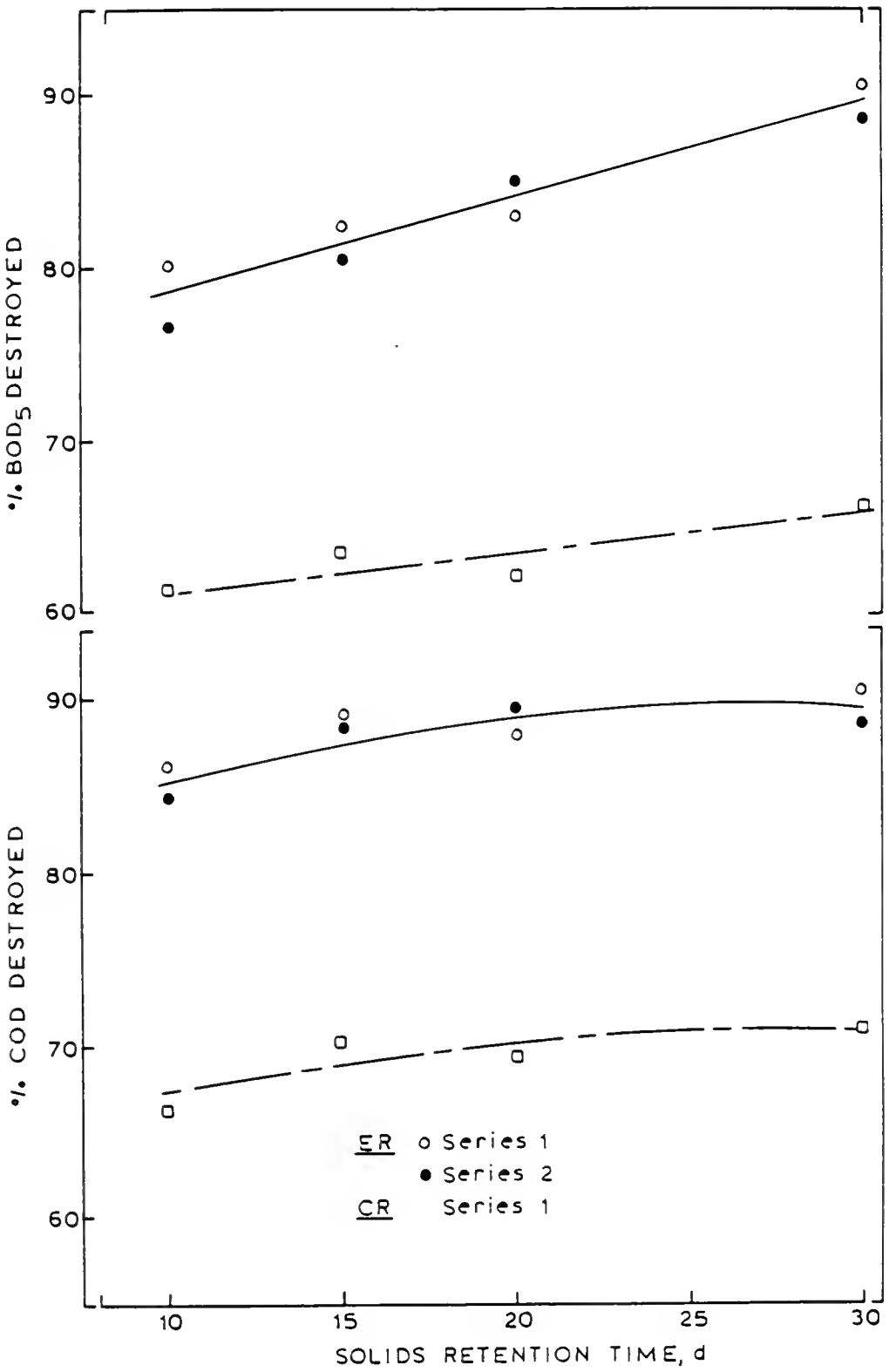

Figure 4-20. Soluble COD and soluble BOD removals related to solids retention time.

Table 4-4. COD available for biomass production.

Parameter	Reactor	SRT 10	15	20	30
Methane gas L/d	ER	0.297	0.606	0.566	-
	CR	0.431	0.632	0.526	0.454
COD equiv. of CH_4, g	ER	0.849	1.73	1.62	
	CR	1.23	1.81	1.50	1.30
COD destroyed g/d	ER	5.629	3.556	2.718	2.030
	CR	4.427	2.939	2.174	1.635
COD available for cell synthesis	ER	4.78	1.826	1.098	2.030
	CR	3.197	1.129	0.674	0.335

4.4.3. Nitrogen and Phosphorus

Nutrient uptake in the ER at steady state is shown in Figures 4-21 and 4-22. Uptake of soluble P, NH_3-N and soluble Kjeldahl-N increased with SRT. Soluble P uptake was not very great, increasing from an average of 44 percent at 10 days to 46 percent at 30 days (Figure 4-21). In the first series, NH_3-N removals increased from 24 percent at 10-d SRT to 66 percent at 30-d SRT (Figure 4-22). In the second series, the NH_3-N removals were a constant 72.5 percent. Soluble Kjeldahl-N removals also differed in the two series, but the removal patterns were similar. In the first series, removals ranged 70-77 percent, while in the second series, the corresponding values were 80-87 percent.

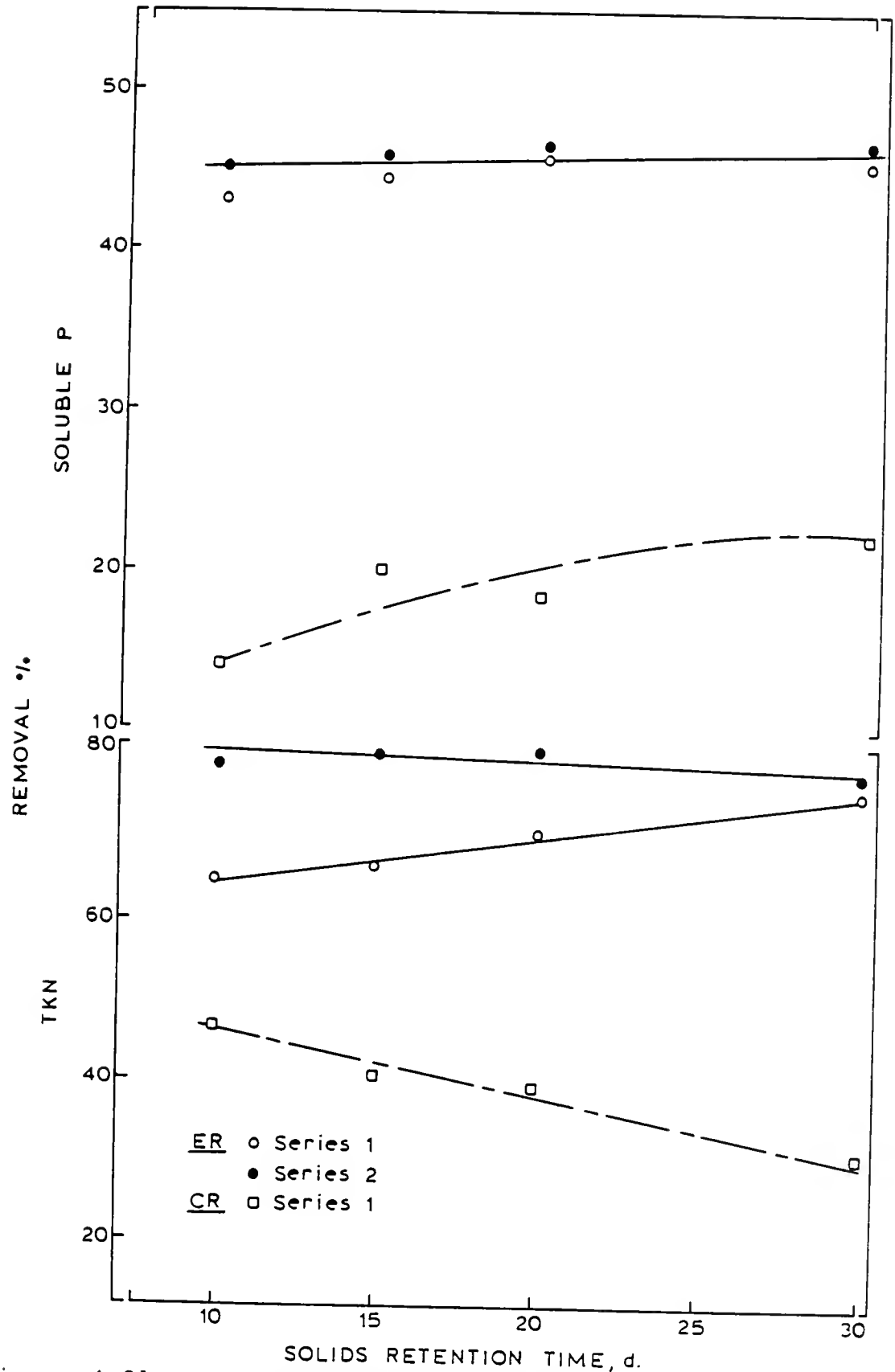

Figure 4-21. Relationship of soluble TKN and soluble phosphorus uptake to solids retention time.

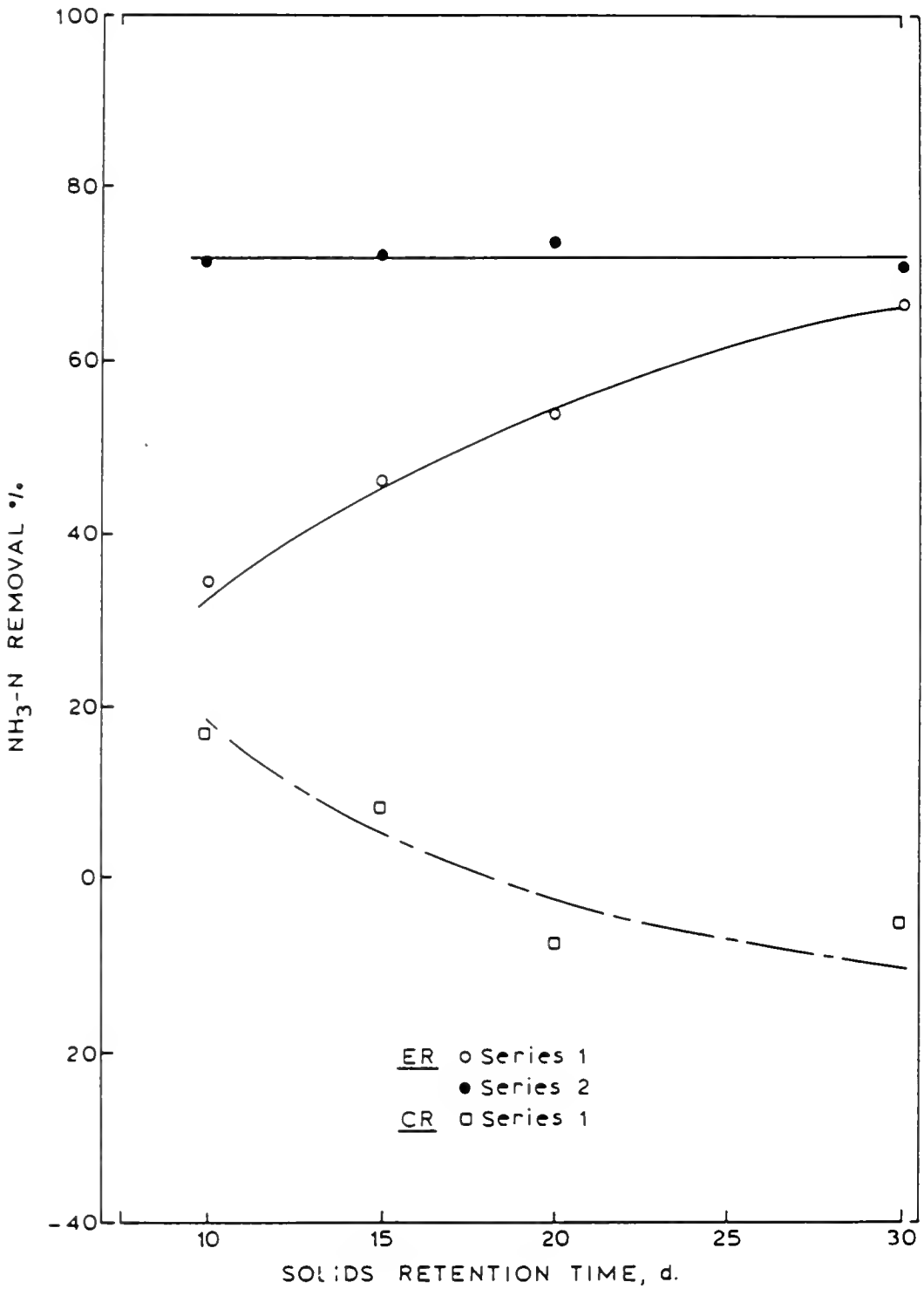

Figure 4-22. Relationship of soluble ammonia uptake to solids retention time.

CHAPTER 5
DISCUSSION

5.1 Substrate Characteristics

The relatively poor conversion efficiency of feed protein to meat protein (25-30 percent) in hogs results in the excretion of waste products containing high levels of protein, amino acids, and urea. In addition, inadequate digestion of the carbohydrate fraction of feed results in excretion of starch and cellulosic materials.

Swine waste is considered a nutritionally well-balanced, easily biodegradable substrate suitable for treatment by anaerobic processes with the formation of methane as a useful by-product (168). Waste characteristics are dependent on a number of factors including feed composition, type and weight of animals, and conditions of housing. In an earlier series of batch studies (45), it was established that purple sulfur bacteria grow well in swine waste and that their presence results in enhanced removals of soluble BOD_5, soluble COD, Kjeldahl-N, ammonia-N, and total P.

The characteristics of the waste collected for these studies from the experimental pig barns of the Swine Research Unit were influenced by the feeding experiment being conducted at the time of collection. Feeding

experiments conducted during these studies had varying levels of protein, phosphorus, potassium, magnesium, trace minerals, and selenium. Trace minerals included calcium, manganese, zinc, iron, copper, and iodine. Selenium levels were, at times, increased to 3.0 percent by weight of feed, this amount being equivalent to 60 times the normally recommended level. Feed components which were not fully metabolized were excreted.

The swine waste used in the laboratory experiments was collected directly from the floor of the pig barns and, being undiluted at the time of collection, contained high concentrations of excreted chemicals which were potentially harmful to microorganisms. In this respect, a flushed, diluted waste would be less hazardous. In Table 3.2 it is observed that the waste characteristics varied over widely. This attributable in part to the growth stage of the hogs at the time of waste collection.

5.2 Bacterial Species in Laboratory Cultures

The dominant species of phototrophic bacteria observed in the laboratory cultures comprised cmall coccoid cells, approximately 1-2.5 μm in diameter, which were identified as Thiocapsa roseopersicina. The dominant species of phototrophs in the lagoon effluent used as inoculum was Thiopedia rosea, a large spherical microorganism, usually 4-6 μm in diameter, which contains gas vacuoles, and stores sulfur

granules inside the cell. Presence of these internal sulfur granules was confirmed in both the inoculum and the laboratory culture. This observation indicated that there was a major shift in bacterial species, from the larger cells growing under natural conditions in the lagoon, to much smaller cells of the same family which proliferated under laboratory conditions. A similar occurrence was observed during laboratory-scale batch studies using effluent from the same lagoon as phototrophic inoculum (45).

Towards the end of the experiments, samples from the laboratory culture and from the lagoon were enriched for identification of the species. Unfortunately, the laboratory sample was taken from the reactor a few days after loading had ceased following termination of an experimental trial. At the time the sample was taken, sulfide would possibly have been depleted, and this therefore accounts for the sulfur-free cells observed by Professor Madigan. The experimental reactor was still being illuminated at the time the sample was taken, and this factor, coupled with a relatively sulfide-free environment, rich in organic compounds, would suggest ideal conditions for growth of the nonsulfur purple bacteria detected in the sample.

Variation in species composition of phototrophic bacteria in natural cultures, and laboratory cultures inoculated from such sources has been investigated (131,187). Early work proposed that light quality might be

an important factor in determining species composition among phototrophic bacteria (176). This was demonstrated to some extent in the laboratory (131) where it was observed that green and yellow light enriched for purple sulfur bacteria, whereas blue light favored green sulfur bacteria.

In a series of continuous culture laboratory studies performed under various light regimes, van Gemerden (187) demonstrated that interspecies competition among the phototrophic sulfur bacteria, and hence species dominance, was influenced by the sulfide affinity of each species, the period of illumination, and the dilution rate. It was observed that in sulfide-limited defined mixed cultures containing two Chromatium species, and subjected to continuous illumination, the smaller Chromatium vinosum species outcompeted the larger Chromatium weissei. Long dark periods coupled with short periods of illumination resulted in a dominant population of the larger-celled Chromatium weissei. van Gemerden observed that immediately following a dark period, the larger C. weissei had a sulfide-uptake rate 2.5 times that of C. vinosum. This rate of uptake rapidly removed a required nutrient from the environment, and under these conditions, the smaller cells could not successfully compete with the larger cells. Occurrence of the smaller-celled Thiocapsa roseopersicina instead of the larger-celled Thiopedia rosea in the laboratory cultures studied during

these investigations would be explained by van Gemerden's observations.

5.3 Impact of Phototrophic Bacteria on the Anaerobic Digestion Process

5.3.1 Gas Quantity and Quality

Successful efficient operation of anaerobic digestion is normally evaluated by the quantity and quality of the gas produced. The volume of methane produced per kilogram of volatile solids or COD added or removed is used as a reference for satisfactory methanogenic metabolism. The quantity and quality of gas produced is dependent on the type and characteristics of the digester feedstock, environmental parameters such as temperature and pH, and the retention time used. Gas production below certain levels is usually indicative of digestion problems.

Phototrophic bacteria metabolize H_2, CO_2, and acetate, the substrates normally used by the methanogens in their metabolic activities and from which most of the methane is produced. In addition, the phototrophs utilize H_2S, a product of anaerobic metabolism which could be inhibitory to the methanogens.

The methanogens and the phototrophs of natural aquatic environments, such as ponds and lakes, are normally spatially separated, the phototrophs locating in the water column where they have access to light and H_2S gas released

from the sediments, while the methanogens are normally found in bottom muds, where there is a more highly reduced environment. Competition for substrates between these two species of anaerobes would therefore not be a critical factor in their interrelationships.

In the completely mixed reactor used in these experiments, the two species are brought into intimate contact by the mixing process, and interspecies competition becomes a factor of greater importance. Such competition could be both beneficial and detrimental to the organisms involved, and survival will be dependent largely on metabolic rate and, ultimately, growth rate of each organism. Under these conditions, the schematic diagram illustrated in Figure 5-1 could be used to describe the interspecies interrelationships which exist in an illuminated anaerobic ecosystem.

In these investigations, the impact of the phototrophs was reflected in the lower gas production rates recorded for the illuminated reactor in which the phototrophs flourished, compared with that of the control reactor which was not illuminated, and which had no significant population of phototrophic bacteria. In experiments in which gasification occurred, the quantity of gas produced at steady state was lower in the ER than in the CR. However, the quality of the gas from the ER was better than that from the CR. The peak gas production rate of the CR, 1.066 L/d (STP), was 18.7 percent greater than that of the ER. The observed

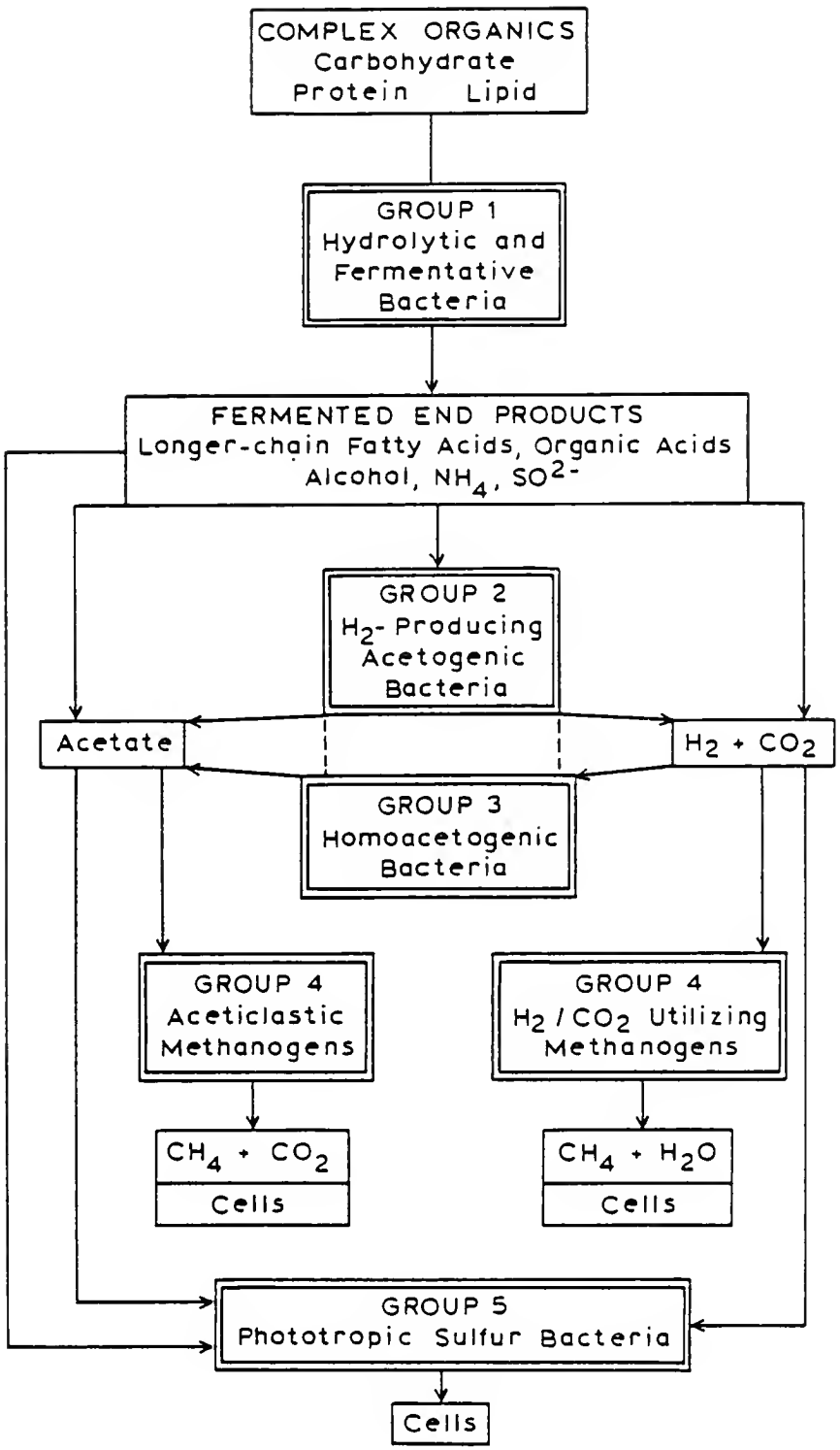

Figure 5-1. Suggested schematic of bacterial interactions during phototrophic anaerobic degradation of organic compounds.

difference was most likely attributable to the assimilation of methane precursors by the phototrophic bacteria. Methane content of the reactor gas was 64-71 percent for the ER and 53-70 percent for the CR. The net result of the difference in gas quality in the two reactors is that the volume of methane produced in each was almost identical. Peak volumetric methane production values were 0.18 L/L-d in the CR, and 0.17 L/L-d in the ER, when the reactors were being loaded at a rate of 0.64 g VS/L.

Using flushed screened swine waste in a digester loaded at the rate of 45 g VS/L, Hill and Bolte (66) recorded gas quality of 55.5 percent methane, and average volumetric production of 0.88 L CH_4/L-d. Loading a digester with swine waste at the rate of 60 g VS/L, Fischer et al. (52) achieved gas quality of 59 percent methane and average volumetric methane production rate of 1.36 L CH_4/L-d. When examined in terms of VS added, the results of Hill and Bolte yield methane production of 0.02 L CH_4/L vol-g VS added, and those of Fischer et al. yield a value of 0.02 L CH_4/L vol-g VS added. The corresponding average methane production values obtained from these studies were 0.02 L CH_4/L vol-g VS added, for both the ER and the CR. Phototrophic bacteria therefore do not have an adverse impact on methane production. Apparently, the reduction in overall gas quantity is compensated by the improved quality of the gas.

The very high steady state concentration of phototrophic bacteria in the ER, 66 mg bchl a/L, attained at 30-d SRT, most likely contributed to the lack of gas production in the ER at this retention time. In addition to this, the feedstock contained only 1.2 g VS/d (0.34 g VS/L-d) and 2.38 g COD/d (0.68 g COD/L-d) at this dilution rate. These levels of substrate would be inadequate to support any significant gas production in addition to the high biomass levels observed.

5.3.2 Waste Treatment and Nutrient Uptake

In anaerobic digesters, the degree of organic reduction achieved is not usually very high, and the effluent stream normally requires additional treatment prior to being discharged into receiving waters. From these experiments, it has been confirmed that a high degree of organic removal can be achieved in anaerobic systems incorporating phototrophic bacteria. However, because of the very high initial organic concentrations, it will still not be possible to discharge the effluent into receiving waters without further treatment.

Steady state values of COD reduction ranged from 84 to 91 percent in the illuminated reactor, compared with 66 to 71 percent in the nonilluminated reactor. COD loading was found to have very little effect on removal rates. Reductions of 66 to 75 percent for TKN, 54 to 68 percent for

NH_3-N and 44 to 46 percent for phosphorus were obtained for the illuminated reactor. These levels of nutrient removal exceed those normally attained in conventional aerobic biological waste treatment processes. Corresponding removals in the CR were much below these levels.

By removing the sulfides which would otherwise cause odor problems in the anaerobic digester, or become toxic to the methanogens if allowed to accumulate above 200 mg/L, the phototrophic bacteria assume a positive synergistic role in their environment.

5.4 Kinetic Parameters and Mathematical Model

The minimum biological solids retention time for the phototrophic anaerobes was found to exist between 8.5 and 10 days, indicating maximum specific growth rate between 0.10 and 0.12 d^{-1}, and corresponding with COD loadings between 6.8 and 8.0 g COD/day.

Using soluble COD as substrate parameter, Equation 2-35 was used to determine the reaction rate coefficient K, considering volatile solids as the measure of biomass. The value of K was determined to be 0.200 L/g-day, and the non-degradable portion of the COD was 2.010 g/L. The resulting design equation for COD removal is therefore given by

$$(S_o - S_e)/X_v t = 0.200(S_e - 2.010) \qquad (5\text{-}1)$$

The biomass production coefficients a_1 and a_2 in Equation 2-32 were determined by plotting $(S_o - S_e)/X_v$ vs SRT. Values of 0.0678 and 1.92, respectively, for the acidogenic phase and 0.0542 and 3.25, respectively, for the methane fermentation stage were determined for these coefficients. The resulting equation governing biomass production then becomes

$$X_v = \frac{(S_o - S_e)}{3.25 + 0.0542/\mu} \qquad (5\text{-}2)$$

and the equation governing the observed yield is

$$Y_{obs} = \frac{1}{3.25 + 0.0542/\mu} \qquad (5\text{-}3)$$

5.5 Application of Results to Field Operations

With suitable modifications, the results of these investigations may be applied to the solution of a number of problems which are currently being experienced in the management of livestock and poultry wastes. Foremost among these are the noxious odors which tend to create a nuisance in the communities in which the operations are located. Phototrophic sulfur bacteria are extremely effective in removing most of the odor-causing compounds from wastes. With the knowledge now available from these studies,

effective odor-free anaerobic lagoons can be designed to achieve high rates of organic removal.

The studies have indicated that gas quality is enhanced by the presence of phototrophic bacteria. These organisms also assist in maintaining a well-buffered anaerobic system. Incorporation of these bacteria in an illuminated anaerobic digester would eliminate the need for very high solids concentration in the feedstock, as is now considered necessary for efficient digester operation.

One area of application with great potential is use of phototrophic bacteria in an integrated system of biogas generation and biomass production. These organisms have a protein content of between 70 and 80 percent. Large-scale production in an illuminated anaerobic reactor and subsequent harvesting for use as a protein source certainly appears feasible. Protein productivity of 3-5 g/d was obtained in these studies. Optimization of the process under field conditions could result in even greater yields.

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The kinetics of swine waste assimilation in an anaerobic photobiological reactor were investigated in laboratory-scale studies, utilizing waste generated at the University of Florida's Swine Research Unit as a substrate. The studies were designed to (1) assess the impact of phototrophic sulfur bacteria on the anaerobic digestion process, (2) to determine the kinetic parameters pertaining to uptake of substrate, and (3) to generate data for the design and operation of pilot- and full-scale units which could be used for waste treatment and large-scale biomass production.

From these studies it was concluded that use of photobiological treatment of swine waste is technically feasible and offers long-term potential for exploitation under suitable conditions. Specific conclusions are that

1. Swine waste is treatable by anaerobic photobiological processes, achieving comparable removal of organic compounds, as measured soluble COD and BOD_5, and better removal of nitrogen and phosphorus than is commonly achieved in standard biological waste treatment processes, and producing good

yields of high-protein photosynthetic biomass which may be harvested and used for a variety of purposes.

2. The minimum biological solids retention time (SRT) lies between 8.5 and 10 days, corresponding to specific growth rate between 0.12 and 0.10-day^{-1}, under the experimental conditions in which these studies were conducted.

3. Washout of phototrophic anaerobes under conditions similar to those used in these studies will be experienced at volatile solids loading in excess of 4.1 g VS/day, and COD loading in excess of 7.8 g COD/day.

4. Substrate uptake increases with retention time, but gas production was optimized at 15-days SRT.

5. Gasification is not an essential prerequisite for the achievement of high levels of treatment.

6. Presence of phototrophic bacteria in anaerobic digestion systems enhances organic removals and gas quality, results in lower levels of total gas production, but a higher quality of gas.

7. A mathematical model comprising three equations was developed to describe the process. The equations are

Substrate uptake

$$(S_o - S_e)/X_v t = 0.200(S_e - 2.010) \quad (5-1)$$

Biomass production

$$X_v = \frac{(S_o - S_e)}{3.25 + 0.0542/\mu} \quad (5-2)$$

Observed yield

$$Y_{obs} = \frac{1}{3.25 + 0.0542/\mu} \quad (5-3)$$

6.2 Recommendations for Further Research

In continuation of these studies it is recommended that

1. The design parameters determined in this series of laboratory-scale experiments should be evaluated in pilot-scale investigations under natural and artificial lighting.
2. Field studies should be conducted to determine the start-up requirements and environmental controls required for successful operation of large-scale anaerobic photobiological systems for the treatment of swine waste.

APPENDIX A
RAW DATA

Appendix A-1. 5-d SRT: temperature, pH and bchl *a*.

Day	Reactor Temp °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
0	25.0	25.0	6.97	6.97	16.30	16.30
1	26.5	25.6	-	-	-	-
2	27.8	26.8	-	-	-	-
3	27.0	26.2	6.80	6.75	21.18	9.38
4	27.5	26.6	-	-	-	-
5	28.0	27.7	-	-	-	-
6	27.8	27.4	6.74	6.69	32.67	8.47
7	27.0	26.5	-	-	-	-
8	28.0	28.0	7.01	6.63	38.72	10.89
9	28.0	29.0	-	-	-	-
10	26.5	27.2	7.08	6.82	42.09	10.89
11	26.5	27.0	-	-	-	-
12	26.8	27.2	7.05	6.81	45.08	10.59
13	27.0	27.0	-	-	-	-
14	28.0	27.3	7.09	6.90	51.80	9.68
15	27.0	26.5	-	-	-	-
16	27.5	27.0	7.17	7.03	55.64	11.50
17	27.0	27.0	-	-	-	-
18	26.0	26.6	7.18	7.00	55.89	10.89
19	26.5	27.0	7.16	7.02	58.36	10.89
20	26.5	27.2	-	-	-	-
21	26.5	27.3	7.18	7.04	58.33	10.89

* denotes start of continuous loading phase.

A-1. continued

Day	Reactor Temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
22*	26.0	27.0	7.15	7.00	58.96	10.89
23	27.0	28.0	7.01	6.83	57.33	8.77
24	27.2	27.6	6.89	6.72	46.89	7.87
25	28.0	27.0	6.71	6.70	36.30	6.05
26	26.5	26.8	6.70	6.72	35.09	6.05
27	27.0	26.8	6.59	6.61	30.55	4.24
28	26.5	26.5	6.50	6.55	23.60	4.24
29	26.5	26.5	6.32	6.37	20.57	3.93
30	27.0	26.6	6.25	6.30	18.76	3.63
31	27.0	26.6	6.23	6.27	18.43	4.24
32	28.0	27.0	6.07	6.11	15.43	2.42
33	27.0	27.5	5.92	6.02	12.71	3.03
34	26.5	26.2	5.77	5.84	10.59	-
35	27.0	28.0	5.66	5.72	8.77	1.82
36	28.0	28.5	5.58	5.64	7.87	-
37	27.0	28.0	5.59	5.62	6.96	1.97
38	27.0	27.5	5.53	5.51	6.66	-
39	27.0	26.8	5.63	5.63	5.75	1.82
40	27.0	26.4	5.77	5.75	4.84	-
41	27.0	27.5	5.87	5.81	4.84	1.36
42	27.0	28.0	5.93	5.91	2.42	1.06

* denotes start of continuous loading phase.

Appendix A-2. 5-d SRT: barometric pressure and reactor gas.

Day	Barom Press.	Reactor Gas					
		Production rate mL/h		Quality %			
				ER		CR	
	in Hg	ER	CR	CH_4	CO_2	CH_4	CO_2
0	29.95	-	-				
1	29.92	14.03	11.81				
2	29.89	15.00	12.41				
3	30.00	15.95	11.19				
4	30.00	15.23	8.18				
5	-	15.73	6.74				
6	30.06	19.28	8.83				
7	30.13	20.43	13.76	49	18	45	24
8	30.18	25.36	10.10				
9	30.15	25.36	19.06				
10	30.10	10.62	16.34				
11	30.04	8.33	17.08				
12	30.05	3.88	20.97				
13	30.10	3.05	24.63				
14	30.12	2.45	28.57	45	20	48	28
15	30.10	2.34	22.55				
16	30.02	2.64	16.26				
17	30.03	5.74	16.83				
18	30.10	3.13	11.67				
19	30.25	3.08	4.17				
20	30.24	3.81	6.86				

A-2. continued

Day	Barom Press. in Hg	Reactor Gas: Production rate mL/h ER	Production rate mL/h CR	Quality % ER CH_4	Quality % ER CO_2	Quality % CR CH_4	Quality % CR CO_2
21	30.15	2.83	6.96	48	22	52	32
22*	30.05	0.65	5.22				
23	30.07	16.46	27.63				
24	30.10	16.46	26.46				
25	30.09	14.84	25.15				
26	30.10	12.24	21.84				
27	30.03	11.02	18.78				
28	30.13	8.25	15.70	48	23	54	22
29	30.20	12.39	14.56				
30	30.19	10.11	15.58				
31	30.17	5.34	15.00				
32	30.12	9.07	16.28				
33	30.17	7.91	15.58				
34	-	5.77	6.54				
35	30.07	5.31	8.57	54	18	55	26
36	30.06	9.40	10.60				
37	30.12	8.98	11.84				
38	30.22	6.67	9.25				
39	30.18	0.00	0.00				
40	30.19	0.00	0.00				
41	30.20	0.00	0.00				
42	30.18	0.00	0.00	55	18	56	28

Appendix A-3. 7-d SRT: temperature, pH, bchl *a*.

Day	Reactor Temp °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
0	27.0	27.0	7.01	7.01	8.47	8.47
1	26.0	26.0	-	-	-	-
2	27.5	27.5	6.93	6.83	10.89	7.87
3	27.0	27.5	-	-	-	-
4	27.0	27.0	7.01	6.52	20.57	6.66
5	27.5	27.5	-	-	-	-
6	27.0	27.0	7.06	6.73	30.25	7.26
7	27.0	26.6	-	-	-	-
8	27.0	26.8	7.01	6.73	39.12	7.67
9	27.5	27.0	-	-	-	-
10	27.0	26.0	7.07	6.79	44.77	6.67
11	27.0	27.2	-	-	-	-
12	27.5	27.3	7.03	6.93	50.01	6.67
13	27.0	27.5	-	-	-	-
14	27.0	27.5	7.03	7.04	56.87	8.07
15	27.5	27.5	-	-	-	-
16	27.0	27.0	7.11	7.06	59.29	6.67
17*	27.0	26.5	-	-	-	-
18	27.0	26.5	7.08	7.00	49.61	6.05
19	26.5	27.2	6.93	6.89	41.95	6.05
20	27.5	27.5	6.82	6.82	33.28	4.24
21	27.0	26.5	6.71	6.85	29.65	3.63

* denotes start of continuous loading phase.

A-3. continued

Day	Reactor Temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
22	27.0	27.0	6.66	6.77	26.62	3.63
23	27.0	27.0	6.62	6.79	22.99	2.42
24	27.0	27.5	6.56	6.79	19.97	3.03
25	27.0	27.2	6.52	6.80	16.94	1.82
26	27.2	27.8	6.46	6.79	14.52	1.82
27	27.0	27.0	6.38	6.79	13.31	1.82
28	27.0	27.0	6.29	6.79	12.10	1.82
29	27.5	26.8	6.28	6.77	11.50	1.82
30	27.0	26.4	6.26	6.79	10.29	1.82
31	27.0	26.4	6.30	6.79	8.47	1.21
32	27.0	28.0	6.30	6.76	8.47	1.21
33	27.8	28.0	6.26	6.75	7.27	1.21
34	28.0	27.0	6.22	6.77	6.66	0.61
35	27.5	26.0	6.09	6.79	6.05	0.61
36	27.2	26.2	6.00	6.68	4.24	0.61

Appendix A-4. 7-d SRT: barometric pressure and gas

Day	Barom Press. in Hg	Reactor Gas					
		Production rate mL/h		Quality %			
				ER		CR	
		ER	CR	CH_4	CO_2	CH_4	CO_2
0	30.03	0.00	0.00				
1	29.94	4.95	7.96				
2	30.10	8.25	12.78				
3	-	7.14	11.63				
4	30.09	9.89	11.83				
5	-	14.08	12.45				
6	30.26	14.34	13.77				
7	30.40	15.35	15.12	48	22	44	33
8	30.28	17.94	21.65				
9	30.14	13.75	21.25				
10	30.39	15.96	20.64				
11	30.38	15.31	29.59				
12	30.32	20.36	32.91				
13	30.30	18.33	31.19				
14	30.18	11.75	27.02				
15	30.25	8.54	30.21				
16	30.20	12.20	29.60				
17*	30.22	16.22	37.78				
18	30.10	15.11	42.83				
19	-	11.26	48.05				

* denotes start of continuous loading phase.

A-4. continued

Day	Barom Press.	Reactor Gas					
		Production rate mL/h		Quality %			
				ER		CR	
	in Hg	ER	CR	CH_4	CO_2	CH_4	CO_2
20	-	14.48	52.10				
21	30.08	12.73	49.77	52	20	51	29
22	30.10	15.23	55.45				
23	30.25	13.13	58.13				
24	30.42	11.88	61.88				
25	30.38	13.33	61.80				
26	-	14.42	60.53				
27	-	14.38	61.24				
28	30.19	17.05	60.68	50	18	52	
29	30.10	18.57	59.18				
30	30.12	18.51	60.08				
31	30.10	17.36	60.19				
32	30.02	19.15	63.62				
33	29.94	19.41	56.86				
34	29.82	16.46	61.88				
35	30.05	15.11	62.13	52	20	53	26
36	30.26	12.91	61.90				

Appendix A-5. 8.5-d SRT: temperature, pH, bchl *a*.

Day	Reactor Temp °C		Effluent pH		Bchl *a* mg/L	
	ER	CR	ER	CR	ER	CR
0*	27.0	-	6.72	-	35.20	-
1	27.0	-	-	-	-	-
2	27.0	-	6.63	-	30.25	-
3	27.0	-	-	-	-	-
4	27.0	-	6.63	-	26.62	-
5	27.5	-	-	-	-	-
6	27.0	-	6.60	-	22.99	-
7	27.0	-	-	-	-	-
8	26.8	-	6.46	-	22.59	-
9	26.0	-	-	-	-	-
10	26.0	-	6.18	-	20.57	-
11	27.0	-	-	-	-	-
12	27.8	-	6.08	-	19.97	-
13	27.0	-	-	-	-	-
14	26.5	-	6.06	-	14.92	-
15	26.5	-	6.05	-	14.12	-
16	26.5	-	-	-	-	-
17	26.5	-	6.05	-	11.50	-
18	26.0	-	-	-	-	-
19	26.5	-	5.97	-	9.08	-
20	26.0	-	-	-	-	-

* denotes start of continuous loading phase.

A-5. continued

Day	Reactor Temp. °C		Effluent pH		Bchl *a* mg/L	
	ER	CR	ER	CR	ER	CR
21	26.5	-	5.98	-	7.04	-
22	26.8	-	-	-	-	-
23	27.5	-	5.95	-	6.56	-
24	27.0	-	-	-	-	-
25	27.0	-	5.93	-	5.08	-
26	27.0	-	-	-	-	-
27	27.0	-	5.94	-	4.60	-
28	27.0	-	-	-	-	-
29	27.0	-	5.97	-	3.32	-
30	27.0	-	-	-	-	-
31	27.0	-	5.92	-	2.98	-
32	28.0	-	-	-	-	-
33	26.5	-	5.93	-	2.42	-

Appendix A-6. 10-d SRT--Series 1: temperature, pH, bchl *a*

Day	Reactor Temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
0	25.0	25.0	7.22	7.22	9.68	9.68
1	27.0	27.0	-	-	-	-
2	26.8	27.2	-	-	-	-
3	26.0	27.0	7.20	7.22	16.95	9.46
4	27.0	27.0	-	-	-	-
5	26.5	27.0	7.28	7.22	21.45	9.40
6	27.0	27.6	-	-	-	-
7	27.2	27.5	7.35	7.24	26.02	9.38
8	28.0	28.4	-	-	-	-
9	27.0	27.4	7.38	7.22	32.67	8.47
10	27.0	27.0	-	-	-	-
11	28.0	27.2	-	-	-	-
12	27.5	27.5	7.38	7.18	35.70	8.47
13	28.5	27.8	-	-	-	-
14	26.5	27.0	-	-	-	-
15	26.6	27.0	7.34	7.13	44.77	9.38
16	27.2	27.5	-	-	-	-
17	29.0	28.0	-	-	-	-
18	28.0	27.6	7.30	7.13	45.35	9.08
19*	27.5	27.6	7.22	7.14	45.68	8.47
20	28.0	27.0	-	-	-	-

* denotes start of continuous loading phase.

A-6. continued

Day	Reactor temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
21	26.5	26.0	7.25	7.17	44.03	7.56
22	27.0	26.5	-	-	-	-
23	27.0	27.0	7.24	7.11	43.86	6.05
24	30.0	30.0	-	-	-	-
25	31.5	31.0	7.11	7.02	43.42	5.12
26	29.0	26.6	-	-	-	-
27	29.0	28.0	7.12	6.89	43.50	4.84
28	28.5	28.5	7.03	6.83	35.09	4.84
29	29.0	28.5	-	-	-	-
30	29.0	28.6	6.93	6.77	41.73	3.63
31	30.0	28.4	-	-	-	-
32	29.5	28.4	6.81	6.83	38.68	4.84
33	30.0	27.5	-	-	-	-
34	29.0	27.5	6.74	6.74	37.76	3.72
35	29.0	26.5	-	-	-	-
36	28.0	27.5	6.66	6.70	35.23	3.93
37	26.6	26.2	-	-	-	-
38	26.5	27.5	-	-	-	-
39	26.0	26.0	6.70	6.70	33.72	3.42
40	27.0	28.0	-	-	-	-
41	27.0	27.8	6.80	6.81	30.04	3.63

A-6. continued

Day	Reactor temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
42	28.0	29.5	-	-	-	-
43	27.5	27.8	6.84	6.85	30.34	3.63
44	27.8	28.0	-	-	-	-
45	26.8	27.4	6.87	6.89	28.46	1.51
46	27.0	27.5	-	-	-	-
47	27.0	27.0	6.84	6.85	28.88	0.61
48	27.0	27.4	-	-	-	-
49	27.0	28.0	6.95	6.86	28.53	0.61
50	27.0	27.5	-	-	-	-
51	27.0	28.0	7.03	7.03	28.83	0.61
52	28.0	27.5	-	-	-	-
53	28.0	28.0	7.04	7.05	28.62	0.61

Appendix A-7. 10-d SRT--Series 1: barometric pressure and gas production.

Day	Barom Press.	Reactor Gas					
		Production rate mL/h		Quality %			
				ER		CR	
	in Hg	ER	CR	CH_4	CO_2	CH_4	CO_2
0	30.18	0.0	0.0				
1	30.18	-	-				
2	30.05	3.0	5.67				
3	30.06	13.45	17.09				
4	30.07	16.60	20.43				
5	30.05	15.95	25.00				
6	30.09	15.10	22.65				
7	30.15	8.0	18.74				
8	30.13	12.69	14.23	48	18	48	32
9	30.17	6.59	10.35				
10	30.11	9.38	14.69				
11	30.02	6.49	12.73				
12	29.85	4.69	14.08				
13	29.89	5.19	15.19				
14	29.95	0.67	13.33	52	18	52	28
15	30.01	1.04	12.29				
16	30.08	1.00	11.32				
17	-	0.00	10.80				
18	-	0.00	10.86				

A-7. continued

Day	Barom Press.	Reactor Gas					
		Production rate mL/h		Quality %			
				ER		CR	
	in Hg	ER	CR	CH_4	CO_2	CH_4	CO_2
19*	-	0.00	3.40				
20	-	10.81	19.82				
21	30.07	16.88	24.42	58	23	52	30
22	30.00	24.79	30.00				
23	30.12	25.37	29.07				
24	30.25	30.51	37.17				
25	30.20	35.29	45.88				
26	30.14	28.13	34.38				
27	30.14	28.22	33.11				
28	30.10	28.00	35.16	61	22	52	28
29	30.03	27.92	32.08				
30	30.07	29.28	34.33				
31	30.08	31.49	32.34				
32	30.16	25.05	27.79				
33	30.02	21.82	24.02				
34	29.97	24.11	26.79				
35	30.04	24.36	25.90	60	22	54	29
36	-	22.86	28.16				
37	-	16.42	26.32				
38	30.04	17.87	25.29				

* denotes start of continuous loading phase.

A-7. continued

Day	Barom Press.	Reactor Gas					
		Production rate mL/h		Quality %			
		ER	CR	ER		CR	
	in Hg			CH_4	CO_2	CH_4	CO_2
39	30.09	18.51	24.26				
40	30.06	20.83	29.79				
41	29.97	22.39	28.70				
42	-	21.44	32.99	62	22	53	28
43	30.11	23.19	29.57				
44	29.99	20.20	30.82				
45	29.93	21.86	32.16				
46	29.93	19.42	30.44				
47	30.15	22.53	31.11	64	20	55	30
48	30.23	21.15	32.31				
49	30.21	20.00	31.74	64	22	53	29
50	30.11	19.18	30.93				
51	30.08	21.61	29.13	64	21	53	31
52	30.04	20.40	33.00				
53	29.65	21.62	31.34	65	21	54	29

Appendix A-8. 10-d SRT--Series 2: temperature, pH and bchl *a*.

Day	Reactor Temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
0	25.0	-	7.40	-	4.84	-
1	28.2	-	-	-	-	-
2	27.0	-	7.26	-	7.26	-
3	27.0	-	-	-	-	-
4	27.2	-	7.23	-	13.52	-
5	27.5	-	-	-	-	-
6	27.2	-	7.13	-	20.05	-
7	27.5	-	-	-	-	-
8	28.0	-	7.24	-	27.72	-
9	27.0	-	-	-	-	-
10	27.0	-	7.19	-	33.45	-
11	27.1	-	-	-	-	-
12	27.2	-	7.16	-	36.60	-
13	27.0	-	7.18	-	36.42	-
14*	27.8	-	7.16	-	35.20	-
15	27.5	-	7.16	-	32.02	-
16	27.8	-	7.14	-	31.46	-
17	26.0	-	7.05	-	31.46	-
18	28.0	-	7.02	-	31.46	-
19	26.5	-	7.02	-	31.46	-
20	27.6	-	-	-	-	-

* denotes start of continuous loading phase.

A-8. continued

Day	Reactor Temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
21	27.2	-	7.02	-	33.88	-
22	27.2	-	-	-	-	-
23	27.8	-	6.98	-	33.88	-
24	26.8	-	-	-	-	-
25	26.8	-	6.84	-	36.90	-
26	27.0	-	-	-	-	-
27	27.0	-	6.77	-	38.72	-
28	28.0	-	-	-	-	-
29	27.0	-	-	-	-	-
30	27.0	-	6.71	-	40.02	-
31	27.0	-	6.90	-	40.52	-
32	27.0	-	-	-	-	-
33	27.5	-	6.95	-	39.93	-
34	26.8	-	-	-	-	-
35	27.0	-	6.84	-	38.10	-
36	27.0	-	-	-	-	-
37	27.0	-	6.90	-	38.72	-
38	27.0	-	-	-	-	-
39	27.0	-	6.80	-	36.30	-
40	27.0	-	-	-	-	-
41	26.8	-	6.75	-	35.70	-

A-8. continued

Day	Reactor Temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
42	27.0	-	-	-	-	-
43	27.2	-	6.72	-	35.70	-
44	27.0	-	-	-	-	-
45	27.0	-	6.75	-	35.09	-
46	27.4	-	6.74	-	35.09	-
47	27.2	-	6.72	-	35.50	-
48	27.0	-	6.72	-	35.20	-

Appendix A-9. 15-d SRT--Series 1: temperature, pH and bchl *a*.

Day	Reactor temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
0	25.0	25.0	7.47	7.47	13.60	13.60
1	26.5	26.8	-	-	-	-
2	26.5	26.8	7.50	7.42	20.57	13.31
3	26.5	26.8	-	-	-	-
4	26.0	26.6	7.48	7.36	23.60	12.40
5	26.0	26.6	-	-	-	-
6	27.5	27.8	-	-	-	-
7	28.0	27.4	7.46	7.31	27.53	13.92
8	26.5	26.8	-	-	-	-
9	27.0	26.0	-	-	-	-
10	26.0	26.5	7.43	7.28	35.09	13.31
11	27.0	27.0	-	-	-	-
12	26.8	27.0	-	-	-	-
13	26.8	27.0	7.44	7.26	43.56	14.23
14	27.0	27.2	7.42	7.26	43.56	13.31
15	27.8	27.2	7.40	7.24	39.93	13.31
16*	27.0	27.3	7.36	7.20	39.93	13.31
17	26.5	27.0	-	-	-	-
18	26.5	27.0	7.32	7.22	41.75	12.71
19	27.8	27.8	-	-	-	-

* denotes start of continuous loading phase.

A-9. continued

Day	Reactor temp. °C ER	Reactor temp. °C CR	Effluent pH ER	Effluent pH CR	Bchl *a* mg/L ER	Bchl *a* mg/L CR
20	27.0	27.8	7.26	7.16	39.33	9.68
21	27.8	27.4	-	-	-	-
22	26.6	26.8	7.22	7.13	40.84	9.68
23	26.6	26.6	-	-	-	-
24	26.6	26.8	7.06	7.00	40.54	8.18
25	27.5	27.0	-	-	-	-
26	27.4	27.2	7.12	7.05	41.33	7.26
27	27.0	27.0	-	-	-	-
28	27.5	27.3	7.10	6.99	43.86	6.05
29	27.0	26.8	-	-	-	-
30	27.5	26.4	7.08	6.93	45.68	5.45
31	26.2	26.2	-	-	42.05	-
32	27.0	27.3	-	-	-	-
33	27.0	27.8	7.10	6.98	45.98	6.05
34	27.0	27.6	-	-	-	-
35	28.0	27.5	7.05	6.93	45.68	4.84
36	27.2	27.2	-	-	-	-
37	27.0	27.0	7.06	6.97	48.75	3.03
38	27.0	27.2	-	-	-	-
39	27.8	27.0	7.10	6.93	51.24	3.63
40	27.0	27.0	-	-	-	-

A-9. continued

Day	Reactor Temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
41	27.5	27.3	7.05	6.95	52.64	2.42
42	28.0	28.0	-	-	-	-
43	28.0	28.0	7.01	6.96	52.85	3.63
44	28.0	28.0	-	-	-	-
45	27.5	28.0	6.96	6.94	52.61	2.42
46	26.8	27.0	-	-	-	-
47	27.0	26.5	-	-	-	-
48	27.5	26.9	6.99	6.99	52.61	1.82
49	27.5	27.8	-	-	-	-
50	27.5	27.6	7.02	7.02	52.03	1.21
51	27.5	27.0	-	-	-	-
52	28.0	27.0	-	-	-	-
53	27.0	27.0	7.01	7.00	52.01	1.21

Appendix A-10. 15-d SRT--Series 1: barometric pressure and gas production.

Day	Barom Press.	Reactor Gas					
		Production rate mL/h		Quality %			
				ER		CR	
	in Hg	ER	CR	CH_4	CO_2	CH_4	CO_2
0	30.15	0.00	0.00				
1	30.05	0.00	0.00				
2	29.86	16.69	0.00				
3	29.97	22.27	0.00				
4	30.00	23.13	11.11				
5	30.11	26.04	29.79				
6	30.23	17.98	26.06	56	20	48	32
7	30.13	9.68	14.19				
8	30.09	4.17	9.38				
9	30.10	5.06	11.95				
10	30.16	1.33	15.24				
11	30.19	4.17	16.67				
12	30.17	2.08	14.17				
13	30.15	0.00	16.67				
14	30.08	1.35	17.69	58	22	50	33
15	30.08	4.77	17.95				
16*	30.19	4.76	4.15				
17	30.27	1.96	3.92				
18	30.15	0.41	6.53				

* denotes start of continuous loading phase.

Appendix A-10. continued

Day	Barom Press.	Reactor Gas					
		Production rate mL/L		Quality %			
				ER		CR	
	in Hg	ER	CR	CH_4	CO_2	CH_4	CO_2
19	30.10	6.96	17.83				
20	30.10	8.72	17.45				
21	30.10	11.36	22.27				
22	30.03	25.37	34.44				
23	30.05	31.59	35.23				
24	30.05	29.23	35.00				
25	29.94	35.21	40.21				
26	30.00	35.21	39.58	58	22	53	30
27	30.08	39.79	37.87				
28	30.08	39.13	41.09				
29	30.10	41.20	41.60				
30	30.24	44.12	42.89				
31	30.22	38.96	42.76				
32	30.20	39.18	48.04				
33	30.13	37.64	51.01				
34	30.15	38.62	50.34				
35	30.15	38.92	49.01	60	20	55	32
36	30.11	40.74	47.65				
37	30.11	38.98	45.71				
38	30.08	40.62	48.87				

A-10. continued

Day	Barom Press.	Reactor Gas					
		Production rate mL/h		Quality %			
				ER		CR	
	in Hg	ER	CR	CH_4	CO_2	CH_4	CO_2
39	30.06	41.04	44.79				
40	29.97	37.29	41.46				
41	29.95	41.12	46.97				
42	29.92	42.97	48.39				
43	29.89	36.97	44.65				
44	30.00	41.84	48.57				
45	30.00	40.00	45.38				
46	30.06	38.43	44.74				
47	30.13	39.91	43.74	66	21	60	26
48	30.18	39.96	44.04				
49	30.15	43.46	48.40	68	22	58	28
50	30.10	37.25	45.49				
51	30.04	37.82	47.17	68	20	59	26
52	30.05	39.78	46.07				
53	30.10	38.13	43.54	68	20	60	25

Appendix A-11. 15-d SRT--Series 2: temperature, pH and bchl *a* .

Day	Reactor Temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
0*	26.8	-	7.16	-	45.36	-
1	26.0	-	-	-	-	-
2	26.0	-	7.10	-	45.62	-
3	27.0	-	-	-	-	-
4	27.8	-	7.02	-	45.98	-
5	27.0	-	-	-	-	-
6	26.5	-	6.85	-	44.77	-
7	26.5	-	6.82	-	44.17	-
8	26.5	-	-	-	-	-
9	26.5	-	6.88	-	42.35	-
10	26.0	-	-	-	-	-
11	26.5	-	6.82	-	39.93	-
12	26.0	-	-	-	-	-
13	26.5	-	6.88	-	39.38	-
14	26.8	-	-	-	-	-
15	27.5	-	6.85	-	39.32	-
16	27.0	-	-	-	-	-
17	27.0	-	6.89	-	39.32	-
18	27.0	-	-	-	-	-
19	27.0	-	6.85	-	38.96	-

* denotes start of continuous loading phase.

A-11. continued

Day	Reactor Temp. °C		Effluent pH		Bchl *a* mg/L	
	ER	CR	ER	CR	ER	CR
20	27.0	-	-	-	-	-
21	27.0	-	6.89	-	38.60	-
22	27.0	-	6.89	-	38.66	-
23	27.0	-	6.85	-	38.72	-
24	28.0	-	6.86	-	38.72	-
25	26.5	-	6.90	-	38.70	-
26	26.5	-	6.90	-	38.70	-

Appendix A-12. 20-d SRT--Series 1: temperature, pH and bchl *a*.

Day	Reactor Temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
0	24.5	24.5	7.18	7.18	4.24	4.24
1	26.5	27.0	-	-	-	-
2	26.5	28.0	7.18	6.92	8.47	4.84
3	26.5	26.8	-	-	-	-
4	26.0	26.5	7.23	6.95	14.61	3.79
5	27.8	26.2	-	-	-	-
6	26.8	26.2	7.25	6.99	20.33	4.66
7	27.5	27.6	-	-	-	-
8	26.4	27.0	7.28	7.06	21.63	4.78
9	27.5	27.6	7.34	7.06	34.49	4.72
10	27.0	26.8	7.36	7.08	33.03	4.69
11	26.5	27.6	7.34	7.08	36.00	4.79
12	28.0	27.6	7.38	7.10	40.54	5.42
13	27.0	27.0	7.36	7.10	43.05	5.44
14	26.5	26.5	7.36	7.12	37.36	5.38
15*	27.5	27.0	7.38	7.13	38.42	3.51
16	26.2	27.4	7.36	7.12	41.44	4.85
17	27.0	27.2	7.34	7.14	43.86	4.99
18	27.2	27.2	7.38	7.15	46.74	4.54
19	27.8	27.2	7.37	7.17	50.22	3.97

* denotes start of continuous loading phase.

A-12. continued

Day	Reactor Temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
20	28.5	27.0	7.37	7.16	44.77	3.63
21	27.2	27.2	7.37	7.18	51.28	3.63
22	27.0	27.0	7.38	7.19	52.49	3.63
23	27.0	27.0	7.40	7.20	52.94	3.63
24	27.0	27.0	7.42	7.19	53.54	3.63
25	27.0	26.0	7.38	7.19	52.03	3,63
26	27.0	27.2	7.39	7.19	54.15	3.63
27	28.0	27.6	7.38	7.18	56.27	3.63
28	27.0	27.6	7.38	7.18	52.94	3.63
29	26.5	26.4	7.36	7.18	52.94	3.63
30	27.0	26.8	7.37	7.18	54.75	2.88
31	29.0	27.8	7.39	7.16	47.19	2.72
32	27.0	27.8	7.38	7.14	40.54	2.42
33	26.8	26.8	7.40	7.18	46.89	2.42
34	27.0	26.8	7.38	7.20	55.06	2.42
35	27.0	27.3	7.38	7.20	53.54	2.42
36	27.0	27.0	7.38	7.22	55.36	2.42
37	27.0	26.6	7.38	7.21	53.54	2.42
38	26.0	26.4	7.41	7.18	54.15	2.42
39	27.0	26.8	7.41	7.20	53.85	2.42
40	27.0	26.8	7.41	7.23	53.24	2.42

A-12. continued

Day	Reactor Temp. °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
41	27.8	27.8	7.41	7.22	53.54	1.82
42	27.2	27.5	7.41	7.21	53.50	2.12
43	26.8	27.0	7.40	7.24	53.36	2.12
44	27.2	26.7	7.41	7.23	53.50	2.12
45	27.5	27.8	7.42	7.23	52.15	2.12

Appendix A-13. 20-d SRT--Series 1: barometric pressure and gas production.

Day	Barom Press.	Reactor gas					
		Production rate mL/h		Quality %			
				ER		CR	
	in Hg	ER	CR	CH_4	CO_2	CH_4	CO_2
0	30.14	0.0	0.0				
1	30.27	-	-				
2	30.33	2.22	1.11				
3	30.27	7.62	2.58				
4	30.01	6.59	7.73				
5	30.04	4.64	1.07				
6	29.85	7.71	6.27				
7	29.87	9.70	6.73				
8	30.05	10.48	8.10				
9	30.08	10.17	7.24				
10	30.05	9.17	7.29				
11	29.86	8.44	7.19				
12	29.95	9.20	11.07				
13	29.70	1.52	12.39				
14	29.47	2.35	14.31				
15*	29.48	2.04	13.04				
16	30.00	13.26	17.08				
17	30.13	15.83	17.92				
18	30.10	16.67	15.21				

* denotes start of continuous loading phase.

A-13. continued

Day	Barom Press.	Reactor Gas					
		Production rate mL/h		Quality %			
				ER		CR	
	in Hg	ER	CR	CH_4	CO_2	CH_4	CO_2
19	30.20	17.08	21.04				
20	30.10	13.54	17.71	60	22	58	26
21	30.09	17.29	14.58				
22	29.88	14.38	18.54				
23	29.77	20.93	24.26				
24	29.94	20.51	26.67				
25	30.05	19.06	26.04	68	20	58	30
26	30.08	20.77	30.42				
27	29,87	27.71	33.13				
28	29.75	26.46	33.54				
29	29.86	22.50	31.46				
30	29.94	26.11	27.78				
31	29.93	39.00	36.00				
32	29.78	30.24	32.68	68	21	60	29
33	29.85	29.17	33.96				
34	30.01	31.89	33.54				
35	30.10	34.17	32.92				
36	30.13	37.08	29.58				
37	30.14	35.42	15.42				
38	30.08	35.87	31.96				

A-13. continued

Day	Barom Press.	Reactor Gas					
		Production rate mL/h		Quality %			
				ER		CR	
	in Hg	ER	CR	CH_4	CO_2	CH_4	CO_2
39	29.82	38.13	35.83	72	20	65	28
40	29.96	31.25	36.67				
41	30.09	41.67	36.46	70	20	64	26
42	30.07	35.42	36.46				
43	30.18	34.58	36.46	71	20	65	26
44	30.15	34.58	36.46				
45	30.15	27.50	35.91	72	20	65	20

Appendix A-14. 20-d SRT--Series 2: temperature, pH and bchl *a*

Day	Reactor Temp °C		Effluent pH		Bchl *a* mg/L	
	ER	CR	ER	CR	ER	CR
0	25.0	-	7.40	-	4.84	-
1	28.2	-	-	-	-	-
2	26.0	-	7.30	-	10.12	-
3	26.8	-	-	-	-	-
4	27.0	-	7.20	-	16.07	-
5	27.0	-	-	-	-	-
6	26.6	-	7.13	-	24.22	-
7	26.5	-	-	-	-	-
8	26.8	-	7.16	-	29.50	-
9	26.5	-	7.18	-	35.20	-
10	26.5	-	-	-	-	-
11	26.8	-	-	-	-	-
12	27.0	-	7.16	-	36.48	-
13	27.0	-	-	-	35.15	-
14*	27.0	-	7.18	-	33.88	-
15	27.5	-	7.18	-	31.90	-
16	27.8	-	7.17	-	32.67	-
17	26.0	0	7.19	-	31.46	-
18	28.0	-	7.19	-	-	-
19	26.5	-	7.20	-	32.67	-

* denotes start of continuous loading phase.

A-14. continued

Day	Reactor Temp °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
20	27.6	-	-	-	-	-
21	27.2	-	7.20	-	32.67	-
22	27.2	-	-	-	-	-
23	27.8	-	7.20	-	32.67	-
24	26.8	-	-	-	-	-
25	26.8	-	7.10	-	33.88	-
26	26.5	-	-	-	-	-
27	26.5	-	7.00	-	35.09	-
28	27.0	-	-	-	-	-
29	27.0	-	6.90	-	36.78	-
30	26.5	-	-	-	-	-
31	27.0	-	6.83	-	38.07	-
32	27.2	-	-	-	-	-
33	28.0	-	6.85	-	40.12	-
34	26.8	-	-	-	-	-
35	27.0	-	6.86	-	41.20	-
36	27.5	-	-	-	-	-
37	27.0	-	6.98	-	43.62	-
38	27.0	-	-	-	-	-
39	27.0	-	7.03	-	44.86	-
40	27.0	-	7.05	-	-	-

A-14. continued

Day	Reactor Temp °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
41	26.8	-	7.07	-	45.05	-
42	27.0	-	7.10	-	45.06	-
43	27.2	-	7.03	-	45.12	-
44	27.2	-	7.13	-	45.02	-
45	27.0	-	7.14	-	44.96	-
46	27.4	-	7.16	-	45.11	-
47	27.2	-	-	-	-	-
48	27.0	-	7.21	-	45.12	-
49	27.0	-	-	-	-	-
50	27.0	-	7.22	-	45.62	-
51	27.0	-	-	-	-	-
52	27.0	-	7.23	-	45.24	-
53	27.5	-	-	-	-	-
54	27.0	-	7.19	-	45.40	-
55	27.0	-	-	-	-	-
56	26.8	-	7.16	-	45.36	-

Appendix A-15. 30-d SRT--Series 1: temperature, pH and bchl *a*.

Day	Reactor Temp °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
0	25.0	25.0	6.92	6.92	5.62	5.62
1	27.0	27.5	-	-	-	-
2	27.2	27.5	-	-	-	-
3	27.0	28.0	6.88	6.65	9.85	6.10
4	26.5	26.0	-	-	-	-
5	26.5	26.3	-	-	-	-
6	26.8	26.5	6.94	6.60	11.35	5.60
7	26.5	26.8	-	-	-	-
8	26.0	27.0	7.07	6.56	20.80	3.63
9	26.5	27.2	-	-	-	-
10	26.5	27.5	7.36	6.76	39.63	4.99
11	26.5	27.2	-	-	-	-
12	26.5	27.5	7.46	6.88	45.68	2.42
13	26.5	27.5	-	-	-	-
14	27.0	26.8	7.44	6.86	59.29	1.82
15	27.0	26.6	-	-	-	-
16	27.0	27.0	-	-	-	-
17	27.0	27.0	7.52	6.98	59.90	2.42
18*	27.0	27.0	7.50	6.98	61.11	2.42
19	27.0	26.5	-	-	-	-

* denotes start of continuous loading phase.

A-15. continued

Day	Reactor Temp °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
20	27.0	27.0	7.22	7.01	61.24	1.82
21	27.0	27.8	7.22	7.04	63.22	1.82
22	27.0	27.8	7.34	7.12	58.08	1.82
23	27.0	27.5	7.36	7.12	62.92	1.82
24	26.8	27.0	7.33	6.97	62.57	1.82
25	27.2	27.8	7.32	7.05	62.89	1.82
26	27.0	27.2	7.30	7.05	62.26	2.27
27	27.0	27.2	7.26	7.02	61.30	1.82
28	27.0	27.0	7.24	7.02	64.16	1.82
29	28.0	28.0	7.26	7.00	65.92	1.82
30	27.0	26.0	7.28	6.96	68.55	1.67
31	26.0	26.0	7.34	6.96	74.02	1.21
32	28.0	27.6	7.39	7.10	81.31	1.21
33	28.0	27.0	7.35	7.10	85.13	1.21
34	26.5	27.5	7.42	7.12	82.65	1.21
35	26.0	27.0	7.50	7.12	77.50	1.21
36	27.5	27.8	7.54	7.14	81.95	1.21
37	27.5	27.6	7.51	7.16	74.96	1.21
38	26.5	26.0	7.55	7.10	73.69	0.91
39	27.4	26.8	7.49	7.15	73.08	0.91
40	27.2	27.2	7.52	7.09	72.46	0.91
41	27.0	26.8	7.50	7.09	69.25	0.61

A-15. continued

Day	Reactor Temp °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
42	27.0	26.0	7.48	7.09	70.05	0.61
43	27.0	26.8	7.46	7.11	68.61	0.61
44	27.0	27.0	7.42	7.05	69.88	0.61
45	27.2	26.4	7.39	7.15	68.61	0.61
46	27.2	27.2	7.41	7.13	70.83	0.61
47	27.2	27.5	7.48	7.12	66.70	0.61
48	28.0	27.0	7.42	7.09	66.79	0.61
49	27.0	26,6	7.36	7.07	66.07	0.61
50	27.0	27.0	7.35	7.05	66.79	0.61
51	26.5	26.2	7.36	7.13	67.94	0.61
52	27.5	26.5	7.36	7.09	67.50	0.61
53	27.5	26.8	7.36	7.09	66.50	0.61
54	27.0	26.4	7.28	7.09	66.92	0.61
55	26.5	27.0	7.25	7.13	66.50	0.61
56	27.0	27.0	7.35	7.10	66.10	0.61
57	27.5	27.8	7.28	7.12	65.20	0.61
58	27.5	27.0	7.23	7.13	66.02	0.61
59	26.5	26.0	7.36	7.14	65.90	0.61
60	28.0	27.5	7.33	7.11	65.90.	0.61

Appendix A-16. 30-d SRT--Series 1: barometric pressure and gas production.

Day	Barom Press.	Reactor Gas					
		Production rate mL/h		Quality %			
				ER		CR	
	in Hg	ER	CR	CH_4	CO_2	CH_4	CO_2
0	30.09	0.00	0.00				
1	-	3.81	0.00				
2	-	3.33	5.62				
3	30.12	5.21	6.25				
4	30.24	2.50	2.50				
5	30.30	4.58	4.58				
6	30.14	4.12	4.95				
7	30.00	2.74	2.74				
8	30.36	2.50	6.25				
9	30.36	4.58	7.71				
10	30.23	1.67	9.58				
11	30.20	0.61	9.18				
12	30.10	0.00	6.32				
13	30.04	3.16	21.89			52	33
14	30.09	4.17	17.50				
15	-	3.93	13.28				
16	30.38	0.38	18.46				
17	30.35	0.18	8.44				
18*	30.33	0.00	8.87				
19	30.28	0.00	9.77				

* denotes start of continuous loading phase.

A-16. continued

Day	Barom Press.	Reactor Gas					
		Production rate mL/h		Quality %			
		ER	CR	ER		CR	
	in Hg			CH_4	CO_2	CH_4	CO_2
20	30.25	0.00	13.54				
21	30.20	0.00	15.92				
22	30.15	0.00	24.32				
23	30.19	0.00	18.65				
24	30.19	0.00	19.71				
25	30.20	0.00	26.15				
26	30.50	0.00	20.68			55	28
27	-	0.00	20.00				
28	-	0.00	23.96				
29	-	0.00	31.46				
30	-	0.00	20.00				
31	-	0.00	24.62				
32	30.19	0.00	29.61			56	31
33	30.09	0.00	30.65				
34	29,95	0.00	37.31				
35	30.08	0.00	36.91				
36	30.24	0.00	39.18				
37	30.10	0.00	43.76				
38	30.02	0.00	39,67				
39	30.08	0.00	40.49				
40	30.14	0.00	40.20				

A-16. continued

Day	Barom Press.	Reactor Gas					
		Production rate mL/h		Quality %			
				ER		CR	
	in Hg	ER	CR	CH_4	CO_2	CH_4	CO_2
41	30.06	0.00	41.91			58	28
42	-	0.00	34.41				
43	-	0.00	41.88				
44	30.03	0.00	41.96				
45	30.03	0.00	41.62				
46	30.14	0.00	41.22				
47	29.89	0.00	42.46				
48	29.80	0.00	43.30			60	31
49	29.87	0.00	39.80				
50	29.84	0.00	36.04				
51	29.85	0.00	31.14				
52	29.85	0.00	28.91				
53	29.84	0.00	44.69				
54	29.85	0.00	28.54			68	24
55	29.84	0.00	29,48				
56	29.88	0.00	31.57			70	23
57	29.83	0.00	28.60				
58	29.85	0.00	29.50			71	22
59	29.88	0.00	30.12				
60	29.95	0.00	29.00			70	22

Appendix A-17. 30-d SRT--Series 2: temperature, pH and bchl *a*.

Day	Reactor Temp °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
0*	26.5	-	6.90	-	38.70	-
1	26.5	-	-	-	-	-
2	26.8	-	6.78	-	41.44	-
3	26.5	-	-	-	-	-
4	26.4	-	6.73	-	41.44	-
5	26.6	-	-	-	-	-
6	26.6	-	6.78	-	43.72	-
7	26.8	-	-	-	-	-
8	26.5	-	6.85	-	45.22	-
9	26.6	-	-	-	-	-
10	27.0	-	7.07	-	48.70	-
11	27.0	-	-	-	-	-
12	26.0	-	7.07	-	48.66	-
13	26.6	-	-	-	-	-
14	27.0	-	7.01	-	49.54	-
15	26.6	-	-	-	-	-
16	26.0	-	7.00	-	49.54	-
17	26.2	-	-	-	-	-
18	26.0	-	7.04	-	50.22	-
19	26.8	-	-	-	-	-

* denotes start of continuous loading phase.

A-17. continued

Day	Reactor Temp °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
20	28.0	-	6.88	-	53.24	-
21	26.8	-	-	-	-	-
22	27.0	-	6.89	-	55.40	-
23	27.0	-	-	-	-	-
24	27.0	-	6.87	-	60.44	-
25	27.0	-	-	-	-	-
26	27.0	-	6.96	-	60.46	-
27	27.0	-	-	-	-	-
28	27.5	-	7.02	-	60.24	-
29	27.2	-	-	-	-	-
30	27.0	-	6.98	-	59.32	-
31	27.2	-	-	-	-	-
32	27.0	-	6.99	-	60.56	-
33	27.4	-	-	-	-	-
34	27.5	-	6.88	-	60.78	-
35	27.5	-	-	-	-	-
36	27.5	-	6.79	-	60.60	-
37	27.6	-	-	-	-	-
38	28.0	-	6.76	-	60.62	-
39	27.6	-	-	-	-	-
40	28.0	-	6.75	-	60.62	-

A-17. continued

Day	Reactor Temp °C ER	CR	Effluent pH ER	CR	Bchl *a* mg/L ER	CR
41	27.5	-	-	-	-	-
42	27.0	-	6.73	-	61.74	-
43	27.0	-	-	-	-	-
44	27.0	-	6.78	-	61.74	-
45	27.0	-	-	-	-	-
46	27.0	-	6.78	-	61.74	-
47	27.0	-	-	-	-	-
48	27.0	-	6.75	-	60.50	-
49	26.8	-	-	-	-	-
50	26.0	-	6.72	-	60.50	-
51	26.2	-	-	-	-	-
52	26.0	-	6.73	-	60.50	-
53	26.8	-	-	-	-	-
54	27.5	-	6.73	-	61.30	-
55	27.3	-	-	-	-	-
56	27.0	-	6.73	-	60.24	-

APPENDIX B
STEADY STATE RESULTS

Appendix B-1. Steady state gas quality--Series 1 trials.

SRT	Reactor			
	ER		CR	
d	% CH_4	% CO_2	% CH_4	% CO_2
10	64	20	55	30
	64	22	53	29
	64	21	53	31
	65	21	54	29
	64 ± 0.50	21 ± 0.82	54 ± 0.96	30 ± 0.96
15	66	21	60	26
	68	22	58	28
	68	20	59	26
	68	20	60	25
	68 ± 1.0	21 ± 0.96	59 ± 0.96	26 ± 1.26
20	72	20	65	28
	70	20	64	26
	71	20	65	26
	72	20	65	27
	71 ± 0.96	20 ± 0.00	65 ± 0.50	27 ± 0.96
30	0	0	68	24
	0	0	70	23
	0	0	71	22
	0	0	70	22
	0	0	70 ± 1.26	23 ± 0.96

Appendix B-2. Steady state effluent solids concentration--Series 1.

SRT	TS mg/L		VS mg/L		TSS mg/L	
	ER	CR	ER	CR	ER	CR
10	10132	8748	7050	6736	9484	8467
	9960	9374	7168	6948	9367	8483
	9794	8796	7111	6720	9442	8450
	9977	9190	7098	7050	9450	8467
	9966*	9027*	7106*	6864*	9436*	8467*
	± 138**	± 305**	± 48**	± 162**	± 49**	± 13**
15	8302	6800	6308	4460	7033	4517
	8414	6850	6405	4515	6916	4420
	8460	6835	6316	4543	7052	4613
	8500	6852	6336	4598	6948	4622
	8419	6834	6341	4530	6987	4543
	± 86	± 24	± 44	± 56	± 66	± 95
20	9876	7916	7382	5530	7287	5816
	9722	7882	7336	5912	7362	5722
	9700	7855	7288	5466	7311	5700
	9688	7822	7336	5568	7326	5686
	9747	7869	7336	5619	7321	5731
	± 87	± 40	± 38	± 200	± 31	± 59
30	13985	8408	10836	4950	13320	7450
	13850	8350	10128	4891	12950	6900
	13890	8430	10525	4720	13075	6824
	13885	8400	10515	4732	13238	7058
	13902	8397	10501	4803	13146	7058
	± 58	± 34	± 290	± 106	± 166	± 279

* denotes average values
** denote standard deviation.

Appendix B-3. Summary of steady state effluent solids concentration.

SRT	Reactor	Series	TS mg/L	VS mg/L	TSS mg/l
10	ER	1	9966 ± 138	7106 ± 48	9436 ± 49
		2	12763 ± 129	9708 ± 90	11823 ± 98
	CR	1	9027 ± 305	6864 ± 162	8467 ± 13
		2			
15	ER	1	8419 ± 86	6341 ± 44	6987 ± 66
		2	12439 ± 69	9542 ± 314	11786 ± 53
	CR	1	6834 ± 24	4530 ± 56	4543 ± 95
		2			
20	ER	1	9747 ± 87	7336 ± 38	7321 ± 31
		2	11918 ± 43	8968 ± 176	8374 ± 140
	CR	1	7869 ± 40	5619 ± 200	5731 ± 59
		2			
30	ER	1	13902 ± 58	10501 ± 290	13146 ± 166
		2	13332 ± 110	10177 ± 117	12579 ± 328
	CR	1	8397 ± 34	4803 ± 106	7058 ± 279
		2			

Appendix B-4. Summary of influent solids concentration.

SRT	Series	TS mg/L	VS mg/L
10	1	12.443±0.266	9.1564±0.088
	2	12.725±0.074	9.703 ±0.088
15	1	11.878±0.205	9.625±0.256
	2	12.439±0.369	9.788±0.178
20	1	11.901±0.242	9.050±0.223
	2	13.200±0.014	10.083±0.020
30	1	13.621±0.312	10.048±0.255
	2	13.332±0.110	10.177±0.117

Appendix B-5. 10-d SRT steady state parameters.

Parameter	Influent		Effluent			
	Series 1	Series 2	Series 1		Series 2	
			ER	CR	ER	CR
pH	6.89 ±0.03	6.97 ±0.02	6.97 ±0.08	6.96 ±0.10	6.73 ±0.02	-
bchl *a* mg/L	0.0	0.0	28.62 ±0.18	0.61 ±0.00	35.48 ±0.29	-
COD* mg/L	19.16 ±0.50	19.07 ±1.00	3.083 ±0.05	6.516 ±0.09	2.304 ±0.06	-
BOD_5* g/L	7.040 ±0.730	6.983 ±0.820	1.408 ±0.078	2.710 ±0.132	1.641 ±0.211	-
TKN* g/L	0.934 ±0.050	0.978 ±0.082	0.331 ±0.022	0.502 ±0.076	0.122 ±0.006	-
NH_3-N* g/L	0.423 ±0.057	0.293 ±0.069	0.279 ±0.078	0.346 ±0.082	0.083 ±0.002	-
P* g/L	0.425 ±0.055	0.470 ±0.076	0.243 ±0.009	0.366 ±0.042	0.259 ±0.011	-

* soluble effluent

Appendix B-6. 15-d SRT steady state parameters.

Parameter	Influent		Effluent			
	Series 1	Series 2	Series 1		Series 2	
			ER	CR	ER	CR
pH	6.98 ±0.02	6.93 ±0.05	7.00 ±0.03	6.99 ±0.03	6.89 ±0.02	-
bchl *a* mg/L	0.0	0.0	52.32 ±0.34	1.67 ±0.58	38.72 ±0.26	-
COD* g/L	18.022 ±0.48	18.484 ±0.62	2.760* ±0.085	5.407* ±0.049	2.102 ±0.035	-
BOD_5* g/L	6.430 ±0.397	7.261 ±0.495	1.125 ±0.102	2.345 ±0.234	1.416 ±0.155	-
TKN* g/L	0.82 ±0.011	0.88 ±0.007	0.26 ±0.006	0.49 ±0.059	0.163 ±0.005	-
NH_3-N* g/L	0.425 ±0.006	0.201 ±0.004	0.231 ±0.004	0.391 ±0.056	0.040 ±0.001	
P* g/ L	0.441 ±0.065	0.464 ±0.044	0.245 ±0.023	0.353 ±0.060	0.251 ±0.032	-

* soluble effluent

Appendix B-7. 20-d SRT steady state parameters

Parameters	Influent		Effluent			
	Series 1	Series 2	Series 1		Series 2	
			ER	CR	ER	CR
pH	6.99 ±0.01	7.01 ±0.03	7.41 ±0.01	7.10 ±0.02	7.19 ±0.02	-
bchl *a* mg/l	0.0	0.0	53.33 ±0.12	2.12 ±0.00	45.26 ±0.11	-
COD* g/L	17.988 ±0.401	18.412 ±0.476	2.455 ±0.065	5.615 ±0.523	2.117 ±0.046	-
BOD_5* g/L	6.910 ±0.654	6.630 ±0.434	1.182 ±0.076	2.626 ±0.168	0.928 ±0.068	-
TKN* g/L	0.789 ±0.087	0.885 ±0.096	0.235 ±0.021	0.477 ±0.079	0.172 ±0.006	
NH_3N* g/L	0.386 ±0.030	0.402 ±0.034	0.178 ±0.010	0.417 ±0.066	0.405 ±0.015	
P* g/L	0.385 ±0.021	0.445 ±0.023	0.201 ±0.015	0.315 ±0.042	0.212 ±0.028	-

* soluble effluent

Appendix B-8. 30-d SRT steady state parameters.

Parameter	Influent		Effluent			
	Series 1	Series 2	Series 1		Series 2	
			ER	CR	ER	CR
pH	6.88 ±0.03	6.96 ±0.05	7.32 ±0.02	7.11 ±0.06	6.72 ±0.03	-
bchl *a* mg.L	0.0	0.0	65.91 ±0.57	0.061 ±0.00	60.70 ±0.40	-
COD* g/L	19.683 ±0.641	20.143 ±0.746	2.333 ±0.068	5.708 ±0.143	2.316 ±0.088	-
BOD_5* g/L	7.611 ±0.362	8.188 ±1.248	0.723 ±0.066	2.603 ±0.197	1.024 ±0.055	-
TKN* g/L	0.913 ±0.029	1.002 ±0.067	0.219 ±0.020	0.639 ±0.018	0.220 ±0.006	
NH_3-N* g/L	0.438 ±0.068	0.377 ±0.054	0.148 ±0.006	0.460 ±0.034	0.105 ±0.007	
P* g/L	0.455 ±0.094	0.554 ±0.115	0.247 ±0.028	0.355 ±0.030	0.298 ±0.076	-

* soluble effluent.

APPENDIX C

MISCELLANEOUS TABLES OF RESULTS

Appendix C-1. Calculation of organic removal rates by phototrophic bacteria in ER--Series 1.

Parameter	SRT 10	15	20	30
S_o mg/l	19 165	18 022	17 988	19 683
S_e mg/L	3 083	2 760	2 455	2 333
$S_o - S_e$ mg/L	16 082	15 262	15 533	17 350
X_v mg/L	7 588	6 141	7 336	10 498
$\frac{S_o - S_e}{X_v}$	2.119	2.485	2.117	1.653
$\frac{S_o - S_e}{X_v t}$	0.2119	0.1657	0.1058	0.0551

S_o = influent COD, mg/L
S_e = effluent filtered COD, mg/L
X_v = average steady volatile solids concentration, mg/L
t = SRT, days

Appendix C-2. Biomass productivity related to bchl *a*.

SRT d	Bchl *a* mg/L Series 1	Series 2	Productivity mg/d Series 1	Series 2
10	28.62 ±	35.48 ±	10.02	12.42
15	52.32 ±	38.72 ±	12.19	9.02
20	53.33 ±	43.26	9.33	7.57
30	65.91 ±	60.70 ±	7.71	7.10

Appendix C-3. Soluble BOD_5 and COD removals.

SRT	BOD_5			COD		
	Series 1		Series 2	Series 1		Series 2
	ER	CR	ER	ER	CR	ER
10	80.2	61.5	76.5	86.2	66.0	84.4
15	82.5	63.5	80.5	89.3	70.2	88.5
20	82.9	62.4	86.3	88.0	69.5	89.5
30	90.5	65.8	88.5	90.5	71.0	88.5

Appendix C-4. Bchl a productivity.

SRT	Series	Flow L/d	Bchl a mg/L		Productivity mg/d	
			ER	CR	ER	CR
10	1	0.350	28.62	0.61	10.017	0.213
	2		35.48	-	12.418	-
15	1	0.233	52.32	1.67	12.191	0.389
	2		38.72	-	9.022	-
20	1	0.175	53.33	2.12	9.333	0.037
	2		45.26	-	7.92	-
30	1	0.117	65.91	0.61	7.711	0.071
	2		60.70	-	7.102	-

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BIOGRAPHICAL SKETCH

Jonathan F. K. Earle was born April 12, 1940, in St. James, Jamaica. He pursued undergraduate engineering studies in England, where he received his bachelor's degree in civil engineering from the University of London in June 1965. Following graduation, he joined the Metropolitan Water Board, London, where he completed his training as an apprentice engineer in the design, construction, and management of waterworks systems. His civil engineering experience includes the design and construction of water distribution pipelines and soft earth tunnels, reservoirs, pumping stations, water treatment plants, and other civil engineering works.

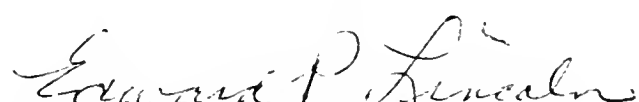
On his return to Jamaica in 1970, he entered the field of consulting engineering and was appointed managing director of the Jamaica branch of the United Kingdom based firm of Howard Humphreys & Sons, international consulting engineers, assuming complete technical and administrative responsibility for all local operations. In 1973 he established the firm of Earle & Associates Limited, Consulting Engineers. This company has since grown to be one of the leading firms in the island in the field of water supply engineering.

Jonathan F. K. Earle is a member of the Jamaica Institution of Engineers, the Association of Consulting Engineers, Jamaica (Past President), the Institution of Civil Engineers (UK), the American Society of Civil Engineers, the American Water Works Association, the Water Pollution Control Federation, and the Royal Society of Health (UK); and a Fellow of the Institution of Water Engineers and Scientists (UK). He commenced graduate studies at the University of Florida in the Fall of 1981 and was awarded a Master of Engineering degree in environmental engineering in August 1983, majoring in water supply and water pollution control. His thesis was entitled "Potential for Utilization of Purple Sulfur Bacteria in the Management of Livestock Wastes." He pursued further research in this field, culminating in the presentation of this doctoral dissertation.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Ben Koopman, Chairman
Associate Professor of
Environmental Engineering
Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Edward P. Lincoln, Cochairman
Associate Professor of
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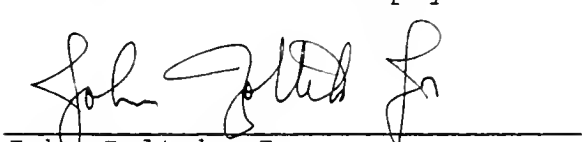

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Engineering and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1985

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